145

TREATMENT OF FERTILIZER INDUSTRY WASTES : A STUDY ON NITRIFICATION

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for the Degree of

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by

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to the

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This is to certify that this work on "Treatment of Fertilizer Industry Wastes: A Study on Nitrification" has been carried out under my supervision and that this has not been submitted elsewhere for a degree.

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ABSTRACT

From a study on nitrification it is found that the nitrification-denitrification system has a tremendous potentiality for the treatment of fertilizer wastes, which, perhaps, cannot be treated by any conventional biological process because of their unique characteristics marked by a high content of ammoniacal nitrogen.

To obtain basic data regarding the factors governing nitrification, controlled laboratory studies were conducted with domestic sewage containing nitrifying seed.

A nitrifying column was used to find out the inhibitory concentration of substrates on nitrifying bacteria and also to find out the effect of pH on these microorganisms. The inhibitory concentrations for Nitrosomonas are 1400 mg/l (0.10 M) NH_3-N and 1100 mg/l (0.0799 M) NO_2^--N and for Nitrobacter, 964 mg/l (0.069 M) NO_2^--N.

Nitrification has been found to occur at extreme pH values like 5.5 and 9.5. The optimum ranges of pH are 7.8 to 8.3 and 8.0 to 8.5 for Nitrosomonas and Nitrobacter, respectively.

From the study on the continuous flow reactor, it is seen that nitrification best proceeds up to a hydraulic loading of 204 ml/(day)(l) when the ammonia-nitrogen concentration in the influent is 700 mg/l.

It is also found that the substrate concentration, S, in the reactor is independent of the substrate concentration, S_o, in the influent up to a certain value of S_o and this value of S_o is dependent on the flow-rate or hydraulic loading. For flow-rates of 1060 ml/day and 640 ml/day, corresponding values of S_o are 700 mg/l and 950 mg/l respectively and above these values, S is not independent of S_o. The reactor volume in both these cases is 5 liters.

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1. INTRODUCTION

A variety of waste products are discharged by industries. These may be as (1) solid wastes, which are normally dumped on ground, (2) gaseous wastes, which are led into the atmosphere, and (3) liquid wastes, which are discharged into streams and watercourses directly or through the waste collection and disposal system of a city or applied on land. Since streams and other watercourses are used for various other purposes also, the liquid wastes are mostly encountered by us and they constitute what is called as industrial wastes (1). Moreover, out of all these three types of wastes, liquid wastes are becoming most conspicuous because the capacity of various waterbodies to assimilate waste material is fast getting exhausted. Furthermore, the water from streams and lakes is being used in greater quantities and for increasing number of uses which demand greater purity of water.

With the advent of ever increasing chemical plants, the existing treatment processes are modified or new methods have to be developed to ensure a safe and amenable disposal of the wastes. The addition of fertilizer industry to the industrial sector has, perhaps, constituted a unique problem of waste treatment and disposal. As more and more fertilizer plants are coming up, not only in India, but in all parts of the world, proper treatment of such wastewaters is becoming imperative.

For the nutritious nature of its wastes, the problem associated with fertilizer wastewater is the turning over of the oligotrophic nature of the receiving water to an eutrophic one which is undoubtedly harmful for well established reasons. Lackey (2) has enumerated some of the ills while discussing the effect of

fertilization of receiving waters. These are: (1)growth of algae, among which some may be toxic in nature, (2)aesthetic harm, such as an unpleasant appearance, taste and odour in potable water imparted by growing algae and obnoxious odours from algal decomposition, and (3)accumulation of dead organic matter which exerts BOD, thereby taxing the oxygen resources. Apart from these, presence of nutrients such as NH_3 or NO_3^- which may be biologically converted to the latter in a waterbody or in drinking water causes methemoglobinemia in which the oxygen transport system in the human body is affected (3).

Treatment of wastewater from a fertilizer plant requires special considerations because of the fact that it contains an unusually high concentration of ammoniacal nitrogen which perhaps cannot be eliminated by employing the conventional treatment processes which are normally advocated for treating domestic or municipal sewage. Usually, nitrogen is removed from the wastewater when it is synthesized into algal or bacterial protoplasm which in turn is separated from the liquid stream. This approach essentially depends on a balanced substrate and environmental conditions congenial for their growth. But this type of treatment is not feasible for fertilizer wastewaters as factors sustaining synthesis of bacterial or algal growth, viz., carbon source, light, phosphorus etc. may be limiting or absent. Hence, treatment of fertilizer wastewater is not feasible by, say, conventional activated sludge process which requires a carbon source. Similarly, algal synthesis may be very low due to limiting light intensity or CO_2 concentration.

Removal of ammoniacal nitrogen which constitutes the core of the problem can, however, be achieved by any one of the following

three methods:

1. Cation Exchange Process

2. Oxidation Pond or Lagoon

3. Nitrification-Denitrification System

The first of these three treatment devices, namely Cation Exchange process is very costly and the expected recovery of ammonia, it is apparent, will not justify the extra cost required. Hence, this method may be abandoned as being not economically feasible.

The last two processes, however, may prove to be practical and economically feasible although the ultimate choice out of these two processes will come from a detailed design consideration and cost.

Notwithstanding these factors and the fact that little is known about the requirements and behaviour of nitrifying organisms, the nitrification-denitrification system may have a high potential for removal of ammoniacal nitrogen. This assumption finds support in the studies of Jhonson and Schroepfer (4) who found that biochemical nitrification-denitrification system has a good potential for attaining higher removals of nitrogen at a more reasonable economic level.

The present study delves into the possibility of adopting the nitrification-denitrification system. In particular, investigation has been carried out to delineate the fundamental reactions involved in nitrification and factors influencing them.

II. LITERATURE REVIEW AND THEORETICAL CONCEPTS

A. Nitrogenous Constituents of Sewage

The nitrogen cycle which is operating in nature is well established. This nitrogen cycle, as suggested by Fry (5) is shown in Figure 1. In biological treatment processes, however, this nitrogen cycle is slightly modified according to Eckenfelder and O'Connor (6) and this is represented in Figure 2.

In the case of raw sewage, the nitrogen content in it is usually found either as organic nitrogen or as free ammonia nitrogen. Delwiche (7) and Bechir and Symons (8) concluded that organic constituents of sewage, exclusive of urea, constitute normally 10% of the total nitrogen. Of this 10%, all species of nitrogenous compounds from amino acids through proteins including the protoplasmic nitrogen of a large and various types of microflora can be expected. Hydrolysis of proteins and other complex nitrogenous compounds and the final decomposition of lower molecular weight residue resulting therefrom will be responsible for the presence of varied nitrogenous compounds. For the sake of analysis, Delwiche (7) has preferred to classify these complex nitrogenous compounds as follows: (1)amino acids, (2)purines and pyrimidines, and (3)other nitrogenous compounds of lower molecular weight. The degradation of these compounds will, however, be functionally dependent upon whether conditions are aerobic or anaerobic. The variation of pH of the reaction and the different intermediates resulting from such degradation will no doubt play a key role in such a treatment process, whether aerobic or anaerobic.

Domestic sewages have been analyzed by a number of workers. Table 1 represents the values reported by Babbitt and Baumann (9).

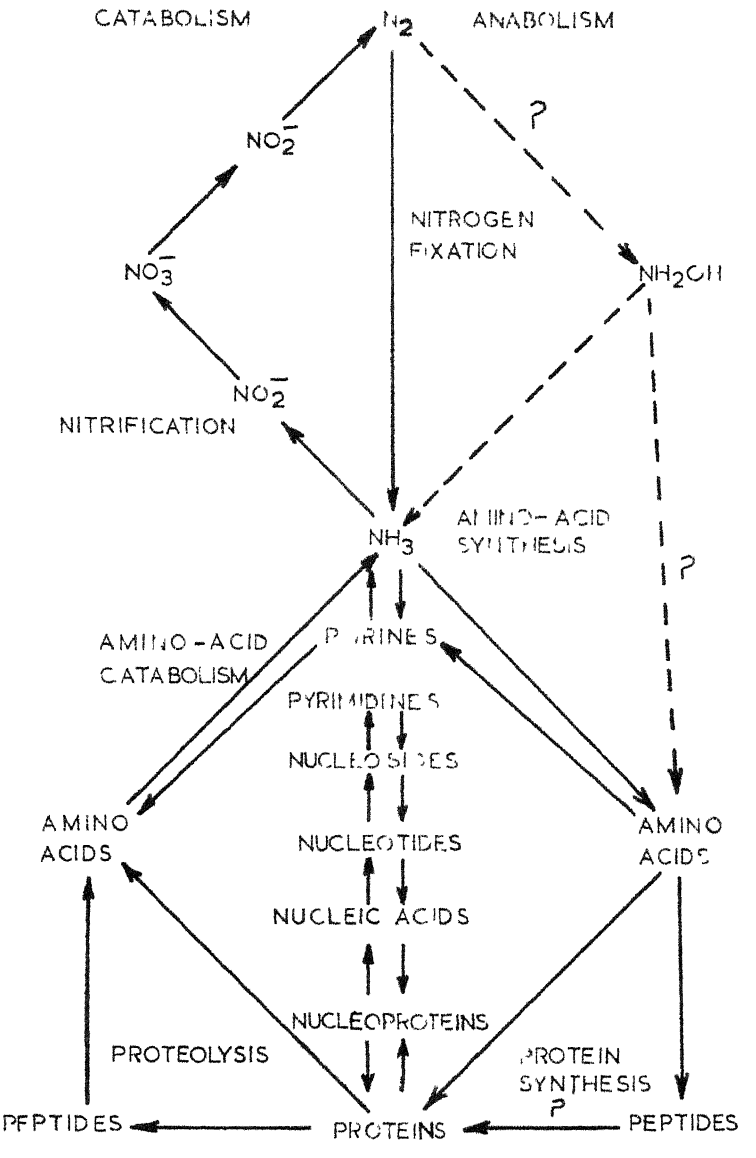


FIG 1 THE NITROGEN CYCLE, AFTER FRY (5)

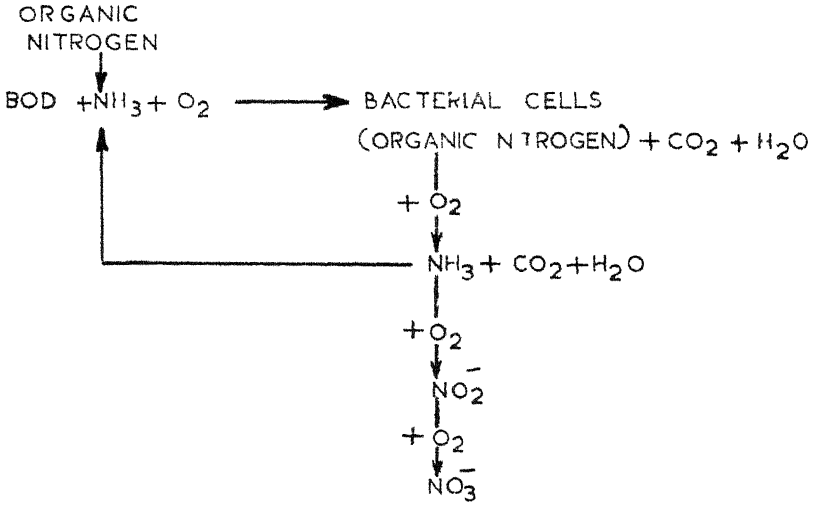


FIG 2 NITROGEN CYCLE IN BIOLOGICAL WASTE TREATMENT, AFTER ECKENFELDER AND O'CONNOR (6)

TABLE 1

TYPICAL SANITARY CHEMICAL ANALYSES OF SEWAGE

(Nitrogen Compounds only)

Constituent	Strong, mg/l	Medium, mg/l	Weak, mg/l
Nitrogen, total	85	50	25
organic	35	20	10
free ammonia	50	30	15
nitrites (RNO_2)	0.10	0.05	0
nitrates (RNO_3)	0.40	0.20	0.10

For Indian cities, Gharpure (10) has suggested different values of nitrogenous constituents in domestic sewage and these values are shown in Table 2.

TABLE 2

ANALYSIS OF SEWAGE

(Nitrogenous Constituents only)

Particulars	Places in India			
	Poona	Kanpur	Lucknow	Allahabad
Free & Saline Ammonia	2.65 ppm	2.43 ppm	6.24 ppm	3.2 ppm
Albuminoid Ammonia	0.65 ppm	1.80 ppm	2.30 ppm	1.5 ppm
Nitrite Nitrogen	nil	nil	nil	nil
Nitrate Nitrogen	nil	nil	nil	nil
Total Nitrogen	3.30 ppm	4.23 ppm	8.54 ppm	4.7 ppm

Conventional treatment processes like activated sludge process, trickling filter etc. have been found to treat domestic sewage containing above mentioned concentrations of nitrogenous compounds quite satisfactorily and the rate of nitrification occurring in these

processes is appreciable. But, however, since fertilizer wastewater contains a very high concentration of ammoniacal nitrogen (in some instances, may be about 200 - 400 times that of domestic sewage), removal of ammoniacal nitrogen from such wastewaters by conventional treatment processes is not feasible.

B. Characteristics of Fertilizer Wastes

Unlike domestic sewage, detailed study on the characteristics of fertilizer wastes have not been carried out so far. However, one may not err much if it is concluded that the wastewater of a fertilizer plant is rather unusual in character, particularly so because of its unique nature of carrying exorbitant amount of ammonia. Nevertheless, it may be worthwhile to consider the expected or estimated characteristics of such a wastewater for formulation of the basic ideas about the types of such wastewaters.

Table 3 shows the characteristics observed in the samples collected from two effluent streams of Sindri Fertilizer Plant, P.O. Sindri, Dhanbad, Bihar (11).

The Urea Fertilizer Plant, Panki, Kanpur, Uttar Pradesh is expected to discharge wastewater of slightly different characteristics which are shown in Table 4.(12).

From these two tables, it is apparent that ammoniacal nitrogen or free ammonia are major constituents of fertilizer wastes.

In this context, it may be mentioned that in India, emphasis has mostly been given on the production of ammoniacal or nitrogenous fertilizers (13). There are about eight nitrogenous fertilizer plants in India, six plants are under construction and are likely to go into operation by 1971 and another five are in proposals. Figures 3

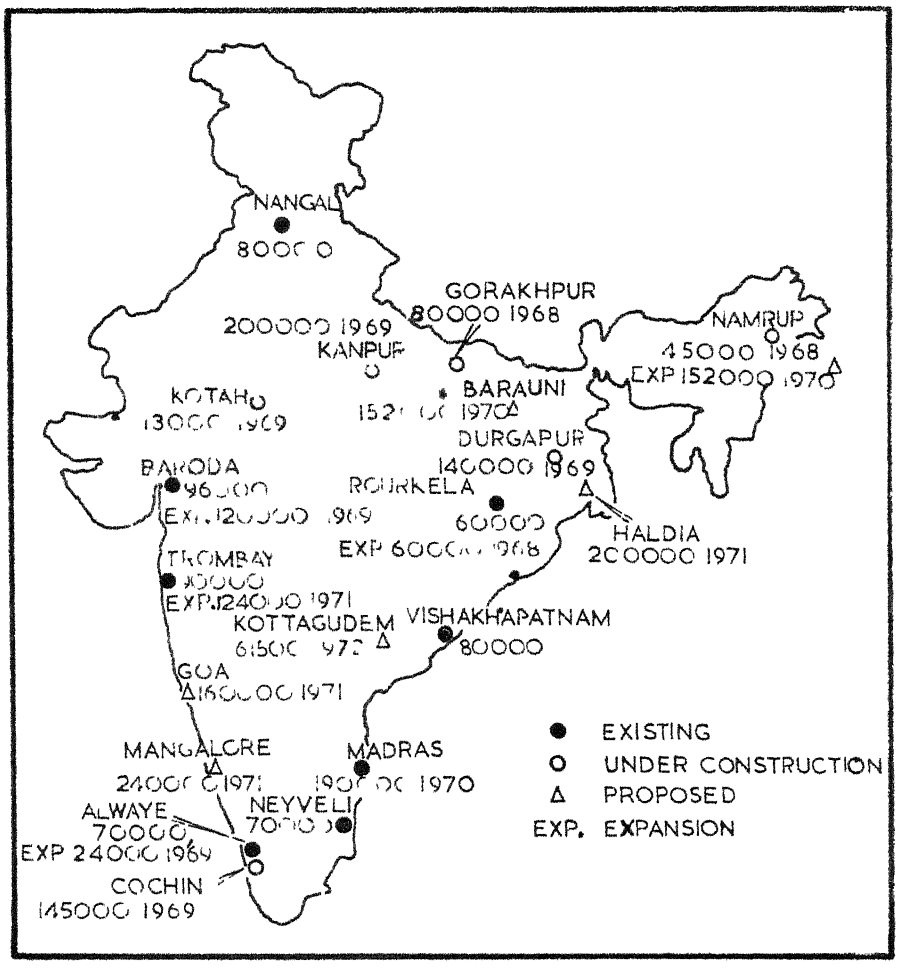


FIG 3 MAJOR NITROGENOUS FERTILIZER PLANTS, AFTER S. LATEEF (14)

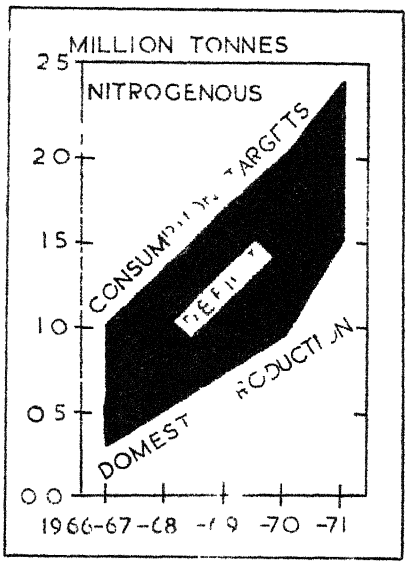


FIG. 4. CONSUMPTION TARGETS AND LIKELY AVAILABILITY OF FERTILIZERS, AFTER S. LATEEF (14)

and 4 show the consumption targets and distribution of various fertilizer plants throughout the country (14).

TABLE 3

CHARACTERISTICS OF WASTEWATER AT SINDRI FERTILIZER UNIT, SINDRI

Description	Period	No. of Reading	Constituents	Analysis in ppm		
				Max.	Min.	Ave.
Stream I	May to June, 1965	15	Total Phenol	8.0	0.2	1.6
			Cyanide as CN	5.0	0.05	1.5
			Amm. Nitrogen	238	7.0	88
			Suspended Solids	7190	960	3369
			Oil	24	1.0	8.2
			Flow in mgd	3.2	1.2	2.1
Stream II	May to June, 1965	15	Phenol	14.0	0.1	0.7
			Cyanide	3.2	0.4	1.2
			Amm. Nitrogen	939	342	313
			Suspended Solids	790	97	398
			Oil	29	2.0	11.5
			Flow in mgd	9.8	6.2	7.8

It is perhaps conclusive to remark that for not only present but for the coming future, wastewaters of the fertilizer plants in India will be enormously rich in nitrogenous compounds, especially in ammonia and thus removal of ammoniacal nitrogen may assume a significant role in any treatment process for the fertilizer wastewaters.

TABLE 4

ESTIMATED CHARACTERISTICS OF UREA PLANT, KANPUR

Item		kg/hr	ppm
Volume	107 M^3/hr		
Temperature	45° C		
Urea		114.7	1065
NH_3		149.3	1410
CO_2		200.5	1860
Other dissolved solids		136.4	1280
Total dissolved solids		251.1	2345
Suspended solids		-	-
Total solids		251.1	2345
Ammoniacal Nitrogen			1160

C. Elimination of Ammonia - Nitrogen from the Wastewater

The importance and necessity of removal of ammoniacal nitrogen from the fertilizer wastes are beyond all doubts. But for their rather unique characteristics, the choice of treatment methods is somewhat limited.

Wuhrmann (15) has pointed out that the elimination of eutrophicating compounds of nitrogen is a primary requirement of sewage purification in many localities, in view of the dangers of secondary pollution of watercourses and lakes due to excessive growth of photosynthetic organisms.

Before considering the various possibilities of elimination of ammonia-nitrogen, it is apt to point out that till now no significant studies have been made towards the treatment of

fertilizer wastes. Whatever studies have been carried out so far, most of them are based on removal of nitrogenous compounds from ordinary domestic sewage.

Nevertheless, earlier workers have noticed that most of the nitrogen which is eliminated is of organic nature (about one-half or one-third of it is on account of the suspended solids in the raw sewage). But it is remarkable that the concentration of the total inorganic nitrogen also decreases appreciably. This is particularly noticeable in the case of experiments carried on for long aeration periods in activated sludge systems (15). However, complete elimination of the primary suspended solids in the raw sewage could only account for a nitrogen elimination to the extent of 20 to 30% of that actually observed. Therefore, the probable inference would be that most of the nitrogen removed from the sewage during the activated sludge treatment, perhaps, comes from the dissolved organic or inorganic nitrogen (15).

Many workers have experienced that a substantial portion of the nitrogen compounds eliminated is transferred to the solids of the activated sludge. Complete nitrogen balance of experiments demonstrating striking nitrogen deficits probably points out occurrence of bacterial denitrification in the course of treatment (15).

Sawyer and Bradney (16) have reported biochemical nitrification and denitrification processes occurring in many biological treatment plants like activated sludge process where nitrites or nitrates are associated with the effluent. Snell (17) has demonstrated the subsequent loss of nitrogen gas when nitrate bearing sludges have been added to the anaerobic sludge digesters. The Kraus modification of the activated sludge process has shown high removals of nitrogen

through the addition of a highly nitrified flow to the head-end of the aeration tank.

Barth et al. (18) reported from the bench and pilot scale studies that modified activated sludge process with controlled zones of anaerobiosis can remove 80% of the influent nitrogen. Some of the special recommendations (for example, tapered aeration, step aeration etc.) may prove their selection especially at higher loads.

Heukelekian (19) observed that the presence of organic matter in sewage does not influence the rate of nitrification of sewage. Moreover, nitrification in seeded sewage proceeds at about the same rate as in a mineral solution and with the addition of activated sludge, nitrification proceeds rapidly. Ludzack and Ettinger (20) suggested that processes involving associated nitrification and denitrification have the potentiality for providing better effluents from the standpoints of oxygen demand and nitrogen elimination at lower air requirements in the usual tank capacity. Bringmann et al. (21) reported 77% removal of ammoniacal nitrogen in the form of nitrogen gas by means of biological nitrification and denitrification processes. Wuhrmann (15) in a similar context has pointed out that elimination rate of nitrogen decreases with decreasing aeration periods.

Rohlich (22) cites a couple of methods which could be profitably employed for removal of nitrogen such as, (1)Guggenheim process (a chemical process operating on the principle of ion exchange), (2)Biological treatment method (treatment by activated sludge process accounted for 65 to 90% removals of NH_3-N whereas by oxidation pond, the removal was about 40% only). He has also pointed out that by nitrification-denitrification system, Wuhrmann achieved a removal of

90% for total nitrogen. A conventional plant produced an effluent containing 12 to 15 ppm of total nitrogen which accounted for about 40 to 50% reduction whereas in the microbial nitrification-denitrification process, the final effluent contained only 2 to 4 ppm of total nitrogen showing a reduction of more than 90%.

Waksman (23) has stated that nitrogen cannot be evolved as a gas unless it is present in a nitrite or nitrate form when a large percentage of the nitrogen will break down into N_2, N_2O and NO gases which will finally escape into the atmosphere. McKinney (24) and Conway, Symons and McKinney (25), and Symons (26) have presented the basic concepts of the use of nitrates in stabilizing wastes with the resultant loss of nitrogen as a gas. Ludzack and Ettinger (20) concluded from their studies that increasing nitrification and denitrification in the activated sludge process offer the best means of diminishing effluent nitrogen in the plant.

In summary, processes such as activated sludge utilizing nitrification and denitrification and oxidation pond can remove nitrogen to a considerable extent. However, such removals have been observed for domestic wastes which have an insignificant amount of nitrogen as compared to fertilizer wastes.

D. Role of Microflora

According to Delwiche (7), vigorous nitrifying flora may be expected in wastewaters containing ammonium ion, provided oxygen is readily available. These nitrifying flora are the kingpin of the nitrification process.

The nitrification-denitrification system as the name suggests, comprises of two distinct stages: (1)nitrification and (2)denitri-

fication. For removal of ammoniacal nitrogen, both the processes are essential since this is a two-stage treatment and the second stage is dependent on the first for ultimate removal of ammonia.

Both nitrification and denitrification processes are discussed below.

1.Nitrification

Nitrification may be defined as the oxidation of some more reduced forms of nitrogen (for example, NH_3) to the nitrite or nitrate valence (27). Nitrification again can be distinctly split up in two stages: (1)oxidation of ammonia to nitrite (sometimes called as nitrosofication or the first stage of nitrification) and, (2)oxidation of nitrite to nitrate (known as nitrification or the second stage of nitrification). However, the exact mechanism of these oxidative processes are not exactly known, but it has been found that for sustaining these oxidative reactions, calcium ions are essential (27).

In the first stage of nitrification, the organisms involved are members of the genera: Nitrosomonas, Nitrosospira, Nitrosocystis, and Nitrosogloea (28) (29). The overall energy yielding reaction of Nitrosomonas (which is most common of the genera listed above) is as follows (4):

$$NH_4^+ + 1.5\ O_2 = 2\ H^+ + H_2O + NO_2^- \qquad (1)$$

These nitrifying bacteria are highly sensitive and specific in nature, Moreover, they are strict autotrophs (30).

The second stage of nitrification, on the other hand, is carried out by another specific group of autotrophic bacteria, known as Nitrobacter which, however, cannot oxidize ammonia to nitrite (28). The energy yielding reaction in the oxidation of

nitrite to nitrate by Nitrobacter can be represented as follows (4):

$$NO_2^- + 0.5\ O_2 = NO_3^- \qquad (2)$$

Aleem and Alexander (31) have reported the recognition of two distinct species, Nitrobacter winogradskyi and Nitrobacter agilis.

Existing information regarding the most common species of Nitrosomonas and Nitrobacter is summarized below in Tables 5 and 6 respectively (32).

TABLE 5

NITROSOMONAS EUROPAEA

Taxonomy:

Order - Pseudomonadales

Suborder - Pseudomonadineae

Family - Nitrobacteraceae

Genus - Nitrosomonas

Species - europaea

Cellular characteristics:

Morphology - Rods, almost ellipsoidal.

Motility - Motile, swarm cells. Flagella polar, 4 to 8 μ in length occurring singly or in a mass.

Size - 0.9 to 1.0 by 1.1 to 1.8 μ.

Habitat - Soil, widely distributed.

Cultural characteristics:

Aqueous media with salts - Grows readily at bottom in soft masses around $MgCO_3$.

Silica gel colonies - Small, compact, brownish, well defined.

Physiological characteristics:

Biochemical action

Ammonia - oxidized to nitrite.

Ammonium salts - oxidized to nitrite.

Growth factors

Oxygen - aerobic

Nutrition - Strictly autotrophic; medium must contain ammonium salt such as ammonium sulphate, magnesium carbonate and potassium phosphate.

Inhibition - Chelating agents inhibit formation of nitrite from ammonia.

TABLE 6

NITROBACTER WINOGRADSKYI

Taxonomy:

Order - Pseudomonadales

Suborder - Pseudomonadineae

Family - Nitrobactereraceae

Genus - Nitrobacter

Species - winogradskyi

Cellular characteristics:

Morphology - Short rods.

Motility - Nonmotile.

Membrane - Gelatinous.

Staining - Gram negative; difficult to stain.

Size - 0.6 to 0.8 by 1.0 to 1.2 μ

Habitat - Soil (alkaline).

Cultural characteristics:

Washed agar colonies - In a week to 10 days, small, circular to irregular, light brown, becoming darker.

Washed agar slant - Scanty, grayish, streak in 7 to 10 days.

Inorganic solution medium - Flocculent, sediment after 10 days; sensitive to ammonium salts under alkaline conditions.

Silica gel - Small dense colonies.

Mineral broth - Sediment in 1 week to 10 days.

Physiological characteristics:

Biochemical action

Nitrate - Formed from nitrite.

Ammonium - Oxidized to nitrites (nitrosofication)

Growth factors

Oxygen - Aerobic.

Temperature - 25 to 28°C.

Nutrition - Strictly autotrophic.

According to Burrows (33), Nitrosomonas europaea is the best known ammonia oxidizer and Nitrobacter winogradskyi is the most commonly encountered nitrite oxidizer. Nitrifying bacteria, although generally soil bacteria, may also be found in fresh water in swamps, streams and lakes (29).

One of the major difficulties in culturing these nitrifying bacteria resides in their extreme sensitivity to excess substrates when in cultures. Meyerhof (34) found that Nitrosomonas is inhibited by its substrate (i.e. ammonia) and Nitrobacter is inhibited by its substrate (i.e. nitrite). It has been seen that there is a sharp substrate concentration optimum for both types of bacteria and it is quite contradictory to the normal behaviour of most other microorganisms with their respective substrates. Moreover,

studies point out that the efficiency of Nitrosomonas decreases as nitrite accumulates whereas growth of Nitrobacter slows down as nitrate accumulates. Again, evidence also suggests that Nitrobacter may be inhibited by free ammonia and, therefore, it will not develop often until Nitrosomonas has lowered the ammonia level.(34). Siddiqi et al. (35) have found that significant inhibition of nitrification occurs at a concentration of 0.10 M ammonia nitrogen. Hofman and Lees (36) have demonstrated that the optimum ion concentration for maximum nitrification is 0.005 M and very definite inhibition occurs at a concentration of 0.10 M ammonia nitrogen.

Various authors have noted the effect of pH on the activity of nitrifying bacteria. Meyerhof (34, found that the optimum pH of Nitrosomonas is 8.5 to 8.8 and that of Nitrobacter about the same (34). Hofman and Lees (36) found that the optimum pH for maximum activity of Nitrosomonas is 8.5 whereas Aleem and Alexander (31) found that Nitrobacter agilis is most active at pH 8.0. Extreme variation of pH to either side (acidic or alkaline), it has been found, may greatly alter or completely suppress nitrification.

Macura (37) found that the method of continuous flow may be applied to the study of some aspects of the metabolic activity of soil microflora including nitrifying organisms. However, according to him, the process of nitrification consists of several phases, in which different groups of microorganisms participate, these being furthermore inequally sensitive towards external factors and, as a result, no steady state can be observed in the study of nitrification. Apart from this, the ratio of the first and second nitrification phase is influenced by ammonia concentration or by the height of soil column.

If a comparison is made with other bacteria, it is found that the nitrifying bacteria are not highly active. A Nitrosomonas cell takes nearly an hour to produce twice its weight of nitrite (34). Moreover, since Nitrosomonas can live only in aerobic conditions, the oxygen consumption is relatively high. As a result of this, the yield of cells per unit of time, or per unit of oxygen consumed, is low.

Various workers have reported several values for the ratio N : C (i.e., NH_3-N oxidized : CO_2 reduced). Winogradskyi (34) found this ratio to be about 35. Employing similar methods, Nelson (34) found this ratio to be 15. On the other hand, Hes (34) found a value close to 53 and for Bömeke (34), between 35 and 53.

It has been concluded from these values that old cultures will give higher figures of this ratio, i.e., they are less efficient in assimilating carbon.

Hofman and Lees (34) have considered all these values of N/C, and have suggested the following equation:

$$\log C = 0.73 \log N - 0.35$$

where C is the organic carbon formed and N is the nitrite formed, both in mg/l. It has been verified by Hofman and Lees (34) that the results of different workers, with different amounts of nitrite formed, are reasonably correlated by this equation.

From this ratio of N : C, thermodynamic efficiency of the nitrifiers can be calculated. For a ratio of 35, Meyerhof (47) found that free energy efficiency of Nitrosomonas is 5.9%. On the other hand, for the value of N/C as 101 for Nitrobacter as obtained by Meyerhof (47), the free energy efficiency is 7.8%. It is evident that young cultures would be much more efficient if the above equation holds good.

While studying solution cultures, Warington (38) established the following facts:

(1) A minute seeding (0.1 gm of soil in a large flask containing sterile ammonium sulphate solution) suffices to start nitrification.

(2) Seedings of soil taken from more than 18 in. in depth were inactive, i.e., the organisms must be only in the upper layers of the soil.

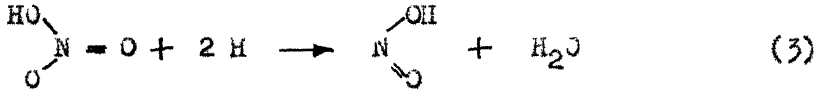
(3) Small amounts of phosphate are needed for nutrition and growth.

Since nitrification is essentially an oxidative process, continuous supply of oxygen or air is insisted upon. From Eqs.(1) and (2), quantity of oxygen required for complete nitrification of ammonium ion (or ammonia) can be theoretically determined which may help in assessing air requirements in any nitrification unit.

2. Denitrification

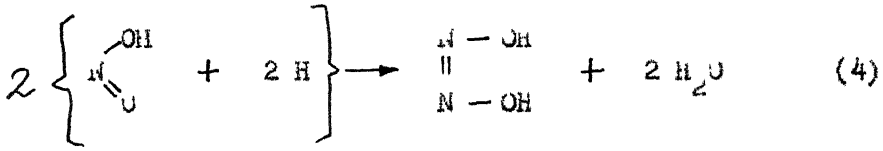
Denitrification may be divided into dissimilatory and assimilatory reductions. The term 'denitrification' usually refers to dissimilatory reduction (27). This term, however, can be applied to the reduction of any form of nitrogen.

Waksman (23) has suggested the following steps in the case of denitrification :



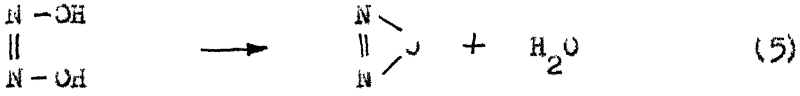
(3)

Nitrate Nitrite



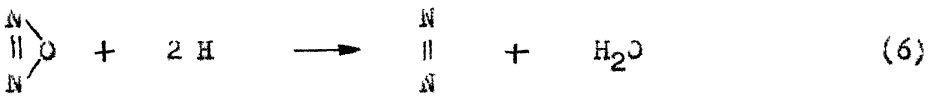
(4)

Hyponitrite



(5)

Nitrous oxide (gas)



(6)

Nitrogen (gas)

The denitrifying organisms reduce nitrate to nitrite or to molecular nitrogen and even to ammonia in substituting either nitrate or nitrite for oxygen as the hydrogen acceptor (39). When nitrite is the end product of the activity of one organism it may either be reduced further, thus completing the denitrification, or may be oxidized back to nitrate by some species other than denitrifying organisms. Reduction to ammonia, however, is a different process from normal denitrification reactions and it has been found that aeration promotes the reduction to ammonia (34). Denitrification, on the other hand, is an anaerobic process in essence.

It has been established by Waksman (25) that the process of denitrification is due to the denitrifying activities of the genus, Pseudomonas. Two organisms have been isolated, one probably Ps. pyocyanea producing N_2 and N_2O, the other, Ps. stutzeri forming mainly N_2. Apart from these two organisms, there is one more distinct species, Ps. denitrifican which is considered responsible for reduction of N_2O to nitrogen gas.

The interesting fact is, perhaps, to note that denitrification may occur to some extent in aerobic conditions although denitrification, as it is, an anaerobic process (34).

E. Reactor Kinetics

Mathematical or rational formulation of biochemical studies is sometimes difficult because of the uncertainty of the actual stages of reactions taking place inside the reactor. But nitrification being

distinctly known, a rational formulation of the reactor kinetics is possible and may be suggestive for striking a balance of the influent and the effluent nitrogen concentrations and thus developing design criteria.

The following mathematical model represents the reactor kinetics which are applicable to a completely mixed reactor in which biochemical reactions are brought about by microorganisms as given by Herbert (40), Aiba et al. (41) etc. on the basis of work by Monod (40).

In a completely mixed single stage reactor of volume V l, in which the substrate which limits the growth rate is being fed at the rate of F l/hr, the mass balance equations regarding the cell concentration X mg/l, product concentration P mg/l and substrate concentration S mg/l can be expressed respectively as follows :

X :

$$V \cdot \frac{dX}{dt} = V\left(\frac{dX}{dt}\right) - FX$$

or,
$$\frac{dX}{dt} = \mu X - DX \qquad (7)$$

where μ is the specific growth rate constant, hr^{-1}, and D is the dilution rate ($D = F/V$), hr^{-1}.

P :

$$V \cdot \frac{dP}{dt} = V\left(\frac{dP}{dt}\right) - FP$$

or,
$$\frac{dP}{dt} = Y_{P/X} \cdot \mu X - DP \qquad (8)$$

where $Y_{P/X}$ is the yield of product per unit cell mass produced.

S:

$$V \cdot \frac{dS}{dt} = FS_o - FS - V\left(\frac{dS}{dt}\right)$$

or,
$$\frac{dS}{dt} = D(S_o - S) - \frac{1}{Y_{X/S}} \mu X \qquad (9)$$

where S_o is the concentration of substrate, mg/l, in the influent and $Y_{X/S}$ is the yield of cellular mass per unit substrate consumed.

At steady-state condition, the left hand side of equations from (7) to (9) is zero and these equations are, respectively, reduced to

$$D = \mu = \mu_{max} \cdot \frac{S}{K_S + S} \tag{10}$$

$$P = \frac{Y_{P/X} \cdot \mu X}{D} = Y_{P/X} \cdot X \tag{11}$$

$$S = S_o - \frac{\mu X}{Y_{X/S} \cdot D} = S_o - \frac{X}{Y_{X/S}} \tag{12}$$

where μ_{max} is the maximum value of the specific growth rate constant and K_S is the saturation constant in Hr^{-1} and mg/l respectively.

To develop design criteria, the above equations can be combined and rearranged as follows :

$$S = \frac{D \cdot K_S}{\mu_{max} - D} \tag{13}$$

$$X = Y_{X/S} (S_o - S) = Y_{X/S} \left(S_o - \frac{D \cdot K_S}{\mu_{max} - D}\right) \tag{14}$$

$$P = Y_{P/X} \cdot Y_{X/S} (S_o - S) = Y_{P/S} \left(S_o - \frac{D \cdot K_S}{\mu_{max} - D}\right) \tag{15}$$

where $Y_{P/S}$ is the yield of product per unit substrate consumed.

($Y_{I/S} = \frac{\Delta P}{\Delta S} = \frac{\Delta P}{\Delta X} \cdot \frac{\Delta X}{\Delta S} = Y_{P/X} \cdot Y_{X/S}$).

The output of cellular mass or product, mg/(l)(hr), can be determined from the following equations respectively.

$$XD = D \cdot Y_{X/S} \left(S_o - \frac{D \cdot K_S}{\mu_{max} - D}\right) \tag{16}$$

$$PD = D \cdot Y_{P/S} \left(S_o - \frac{D \cdot K_S}{\mu_{max} - D}\right) \tag{17}$$

To find the particular value of D at which the output is maximum, D_{max}, the first derivative of equations (16) and (17) is

equated to zero to obtain

$$D_{max} = \mu_{max} \left(1 - \sqrt{\frac{K_S}{K_S + S_o}}\right) \qquad (18)$$

Figure 5 shows a plot of equations (13), (14) and (16) according to Aiba et al. (41) for steady-state relationships in a continuous flow reactor.

Even though for most reactions $K_S \ll S_o$ and the maximum output occurs near the wash out stage, D approaching μ_{max}, Herbert (42) and other workers have pointed out that in practice it is difficult to maintain the steady-state at this level of flow, since a slight change of D would result in a large variation of P, S or X.

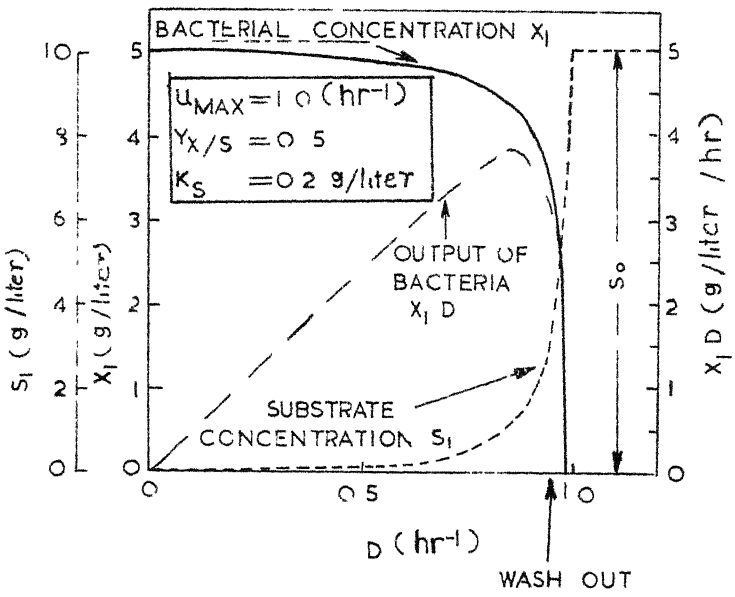


FIG.5 STEADY-STATE RELATIONSHIPS IN A CONTINUOUS CULTURE, AFTER AIBA ET AL. (41)

III. SCOPE OF STUDY AND OBJECTIVE

Various organic and inorganic chemical compounds which are either the raw materials or the end-products of fertilizer industry are potential water pollutants through losses in manufacturing processes or end-use. Proper treatment methods should be ensured before these wastes are finally disposed to a river or stream as they may result in over-fertilization of the latter due to the presence of nitrogen in the wastes. Improved degree of nitrogen removal from such wastewaters, rich in nitrogen content, would be beneficial in helping to control the nuisance and unhealthy conditions resulting from eutrophication of receiving watercourses.

Although it is true that nitrification-denitrification system is yet to achieve popularity in biological waste treatment, it has been pointed out in the last chapter that it has got a high potentiality for treating fertilizer wastes which are very much unusual in nature.

This little acquaintance with the application of such a treatment process, perhaps, gives a scope for a considerable amount of exploratory research and delineation of fundamentals involved. Moreover, significance of new findings in this case may render appreciable improvement to the treatment plant to be designed and also may make the handling of wastewaters easy and efficient both internally and externally.

With whatever information available in this context, the followings have been outlined as the objectives of this study.

A. Inhibitory Concentration of Ammonia

Strictly speaking, inhibition is the word used to designate the taking in of water by protoplasm or gels (43). However, in

general, inhibition means prevention, as a result of the influence of nerve impulses, of activation of an effector (44). In the case of microorganisms, inhibition stops their ability to multiply and also sets back their normal activities. Normally, any growth can be inhibited : (1) by interfering with the energy supply, as by poisoning oxidations; (2) by reducing the supply of materials, especially of the most critical materials, such as vitamins, needed in small amounts and not replaceable; (3) by interfering with the synthetic process itself, especially with its most vulnerable participants, the nucleic acids. Apart from these, the nitrifying bacteria have to fight against inhibition when an excess amount of substrate is fed into them.

Wastewater of a fertilizer plant being excessively rich in ammonia, the influence of ammonia on the nitrifying bacteria may be worth studying. Hence, the first stage of this study was devoted to finding out the inhibitory concentration of ammonia on these bacteria. Undoubtedly, ignorance of this crucial concentration may upset the whole treatment process as the nitrifying bacteria will be rendered absolutely ineffective by this concentration of ammonia.

b. Effect of pH

It is a fact that the pH of a culture medium has an important effect on the number of survivors. Most organisms are most easily killed in acid or alkaline solutions than in a neutral or nearly neutral environment. Moreover, changes in the pH of an enzyme system often profoundly alter the reaction rates. In fact, enzymes are as sensitive to alterations in pH as they are to temperature or even more so. They have definite minimal, optimal and maximal zones and limits in respect to pH, just as they do to temperature (28).

Since whatever is applicable to enzymes is largely applicable to living cells, the reaction of bacteriological culture media must, therefore, be very carefully adjusted with respect to pH.

An optimal pH range exists for any enzyme, or for that matter, for any culture, This pH value is, however, not fixed but varies with the state of purity of the enzyme, its biological source, the temperature, and the nature and concentration of the salts present (39).

In the last chapter it has been shown that for Nitrosomonas and Nitrobacter, the optimal pH values are different. Moreover, there is a scope for ambiguity for their optimal pH values as various workers have arrived at different values.

The second part of this study is, therefore, devoted to finding out the relationship of nitrification with respect to pH and to determine the optimum pH values for both types of nitrifying organisms.

C. Nitrification in a Completely Mixed Continuous Flow Reactor

It is not unlikely that the rate of nitrification found in batch processes and similar bench-scale and pilot-plant studies may be different from that of a continuous flow and completely mixed reactor. Nitrification, as has been shown earlier, is essentially an aerobic process and depends on a large number of factors such as, amount of nitrifying flora present, rate of aeration, concentration of substrate, various operating conditions (viz., temperature, pH etc.) etc.

Suggesting a treatment device without proper design data is of little significance. In any biological waste treatment, design of the plant is mostly dependent on such factors as the shape and

size of the unit, optimum detention time or hydraulic loading, and substrate loading.

For developing the design of nitrification-denitrification system, the following factors were studied :

1.Effect of hudraulic loading

2.Effect of substrate concentration

Investigations pertaining to shape and size of the reactor and oxygen consumption were, however, not considered.

IV. MATERIALS AND METHODS

A. Experimental Design and Equipment

Heukelekian (19) concluded that oxidation of carbonaceous materials does not inhibit or retard nitrification which can take place simultaneously, provided that : (1) the oxidation of carbonaceous materials does not create a deficiency of oxygen, and (2) there is an active nitrifying flora. In literature review also it has been discussed that some factors and operating conditions may directly affect nitrification, for example, (1) supply of oxygen, (2) supply of substrate (ammonia, in this case), (3) proper pH value, (4) supply of required carbon dioxide etc.

While planning the experiments, due consideration was given to all these various aspects. Experiments were conducted using both batch and continuous flow operations in laboratory reactor units. Solution of ammonium chloride (NH_4Cl) was used to assess nitrification.

1. Source of nitrifying bacteria

The nitrifying bacteria which are almost always found in domestic sewage, finding their way from soil, were cultured by aerating the domestic sewage. Domestic sewage is usually a good source for both types of nitrifying bacteria.

For all experiments in this study, nitrifying seed was developed by aerating domestic sewage for a few days after adding an inorganic medium (described in the following section), substrate and buffer (to keep the pH at the desired level). When sufficient number of both *Nitrosomonas* and *Nitrobacter* developed as indicated by the accelerated rate of nitrification, the sewage was utilized as the source of nitrifying bacteria.

2. Inorganic medium

Since both Nitrosomonas and Nitrobacter are strict autotrophs, they thrive only in inorganic medium and cannot utilize organic compounds, some of which may even prove to be inhibitory to their activity. In essence, autotrophs synthesize complex compounds, composing their protoplasm, from simple inorganic salts and utilize carbonates or carbon dioxide as their carbon source (29).

Table 7 gives the details of the inorganic medium used by Siddiqi et al. (35). The same medium was provided in all the experiments in this study.

TABLE 7

INORGANIC MEDIUM

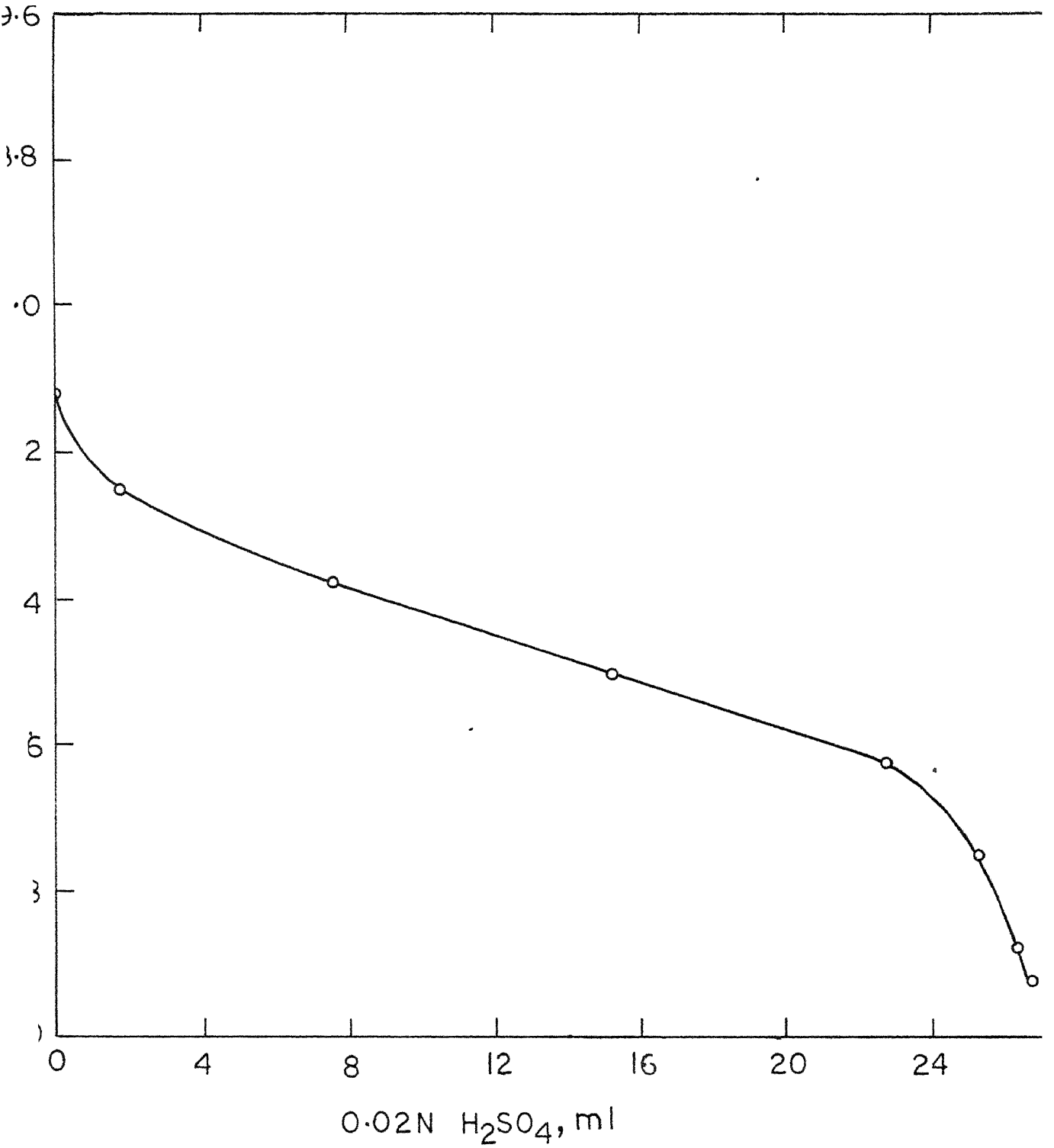
$FeCl_3 \cdot 6\ H_2O$	0.125 mg/l
$MgSO_4 \cdot 7\ H_2O$	25.0 mg/l
K_2HPO_4	3.0 mg/l
$CaCO_3$	50.0 mg/l
$NaHCO_3$	250.0 mg/l
NH_4Cl	As desired

The medium, although contained $CaCO_3$, had little buffering capacity as shown in Figure 6. Hence, a separate buffer was always added for keeping pH at the desired level.

3. Inhibitory concentration of ammonia

To find out the particular concentration of substrate at which it becomes inhibitory to the nitrifying bacteria, several tests were run in a nitrifying column, shown in Figure 7.

The nitrifying column was mounted on a wooden stand and it



6. BUFFERING CAPACITY OF INORGANIC MEDIUM, 50ml VOLUME

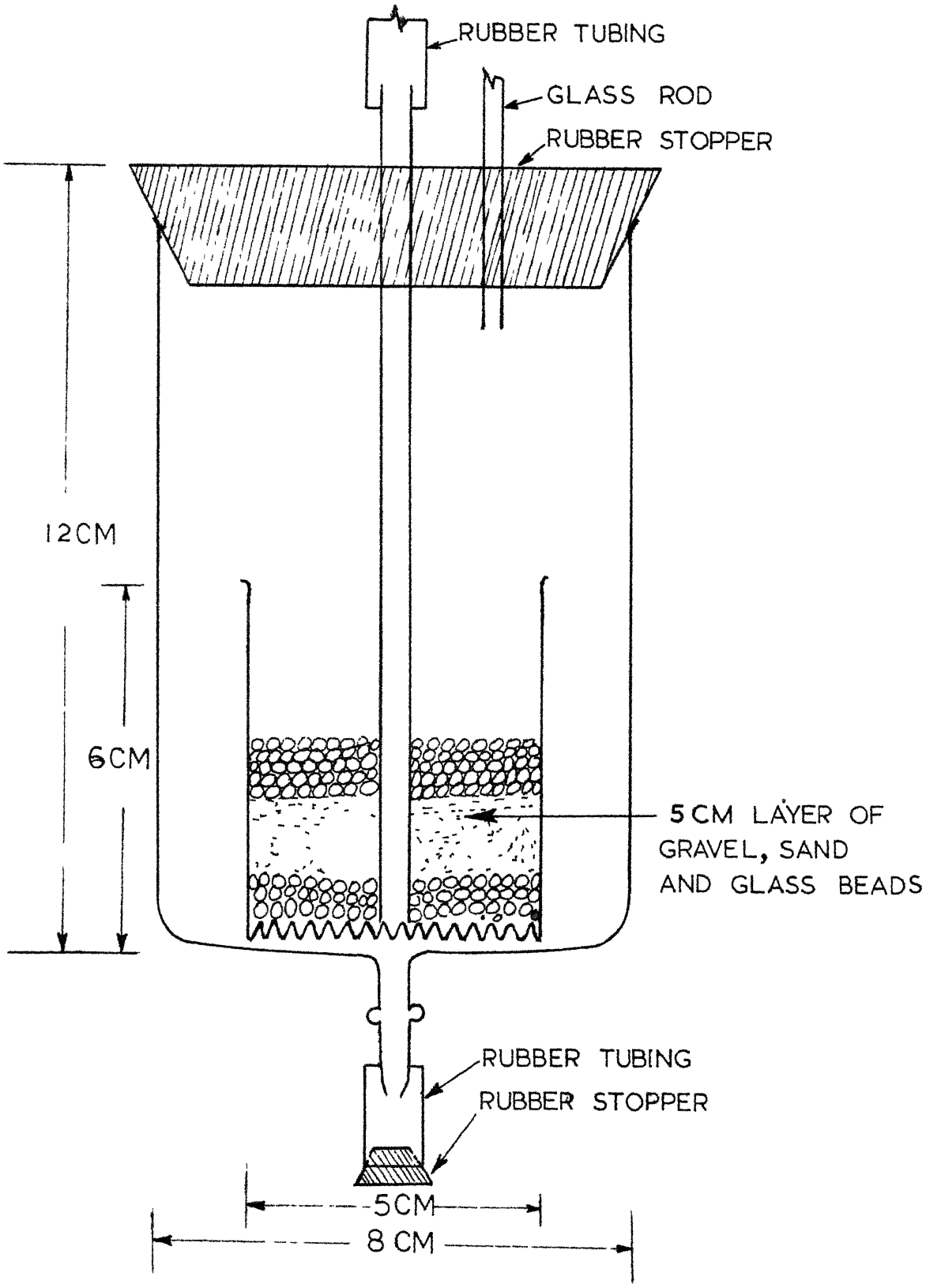


FIG. 7. DETAILS OF NITRIFYING COLUMN

was aerated by bubbling air through it. At the bottom of the inner column, some holes were provided for recirculation of the contents. The column was packed with coarse sand supported on gravel and at the top of this layer, glass beads were provided to resist lifting of sand particles.

The column was filled with sewage containing nitrifying flora. To this, sufficient quantity of inorganic salts to keep the composition same as shown in Table 7 were added and the total volume in the column was made up to 250 ml. Every day, a 50 ml sample was drawn from the column for analysis of NH_3-N, NO_2^--N, NO_3^--N, organic nitrogen and pH and the same volume of feed was added containing ammonia-nitrogen concentration under study. Determination of dissolved oxygen in the column was done from time to time to ensure aerobic condition. The daily feed also contained the same inorganic medium discussed above.

The rate of aeration, depth and position of the sand layer were not disturbed throughout the study. Temperature was found to be fairly constant, varying between 29°C and 30°C.

In the initial stages of this experiment, no buffer was added. But later on, as the pH went down, 1 M NaOH solution was added to raise the pH level and, thereafter, a concentration of 0.1 M phosphate buffer was maintained in the column.

After the column had shown considerable nitrification as evidenced by a great deal of conversion of NH_3-N to NO_2^--N and NO_3^--N, the concentration of ammonia in the column was changed and so also for the subsequent feeds.

The concentration of ammonia was thus increased till there was inhibition of nitrification.

4. Effect of pH

In the nitrifying column described earlier, tests were conducted in a similar fashion to find out the effect of pH on the rate of nitrification.

On the basis of earlier experiments, an optimum concentration of NH_3-N (0.05 M) was chosen for this part of the study. The observations were started with a pH of 8.0. This value was chosen on the basis of previous works by other investigators. Only when an equilibrium was attained, the pH value was changed, firstly, above 8.0 and then, below 8.0. Before changing the pH from a high level to a low level (from 9.5 to 7.5, in this case), the pH was first brought down to 8.0 and the column was maintained at this pH for a few days to achieve adjustment.

The pH at various levels was maintained by using 0.1 M phosphate buffers. To change the pH value, sufficient amount of 1 N NaOH or 1 N KH_2PO_4 was added prior to adding the appropriate buffer.

5. Nitrification in a continuous flow completely mixed reactor

As the discharges in actual operation would be only continuous, this phase of study was devoted to finding out nitrification in a continuous flow and completely mixed reactor.

Figure 8 represents the laboratory set-up of the reactor-vessel along with other accessories. The liquid volume in the reactor was maintained at 5 litre by keeping a constant level of over-flow. In the centre compartment, a bed of gravel, sand, and glass beads was provided and two diffusers were embedded in it for aeration. The centre compartment had holes in it for recirculation of the liquid. The flow rate from the feed-tank was controlled by means of

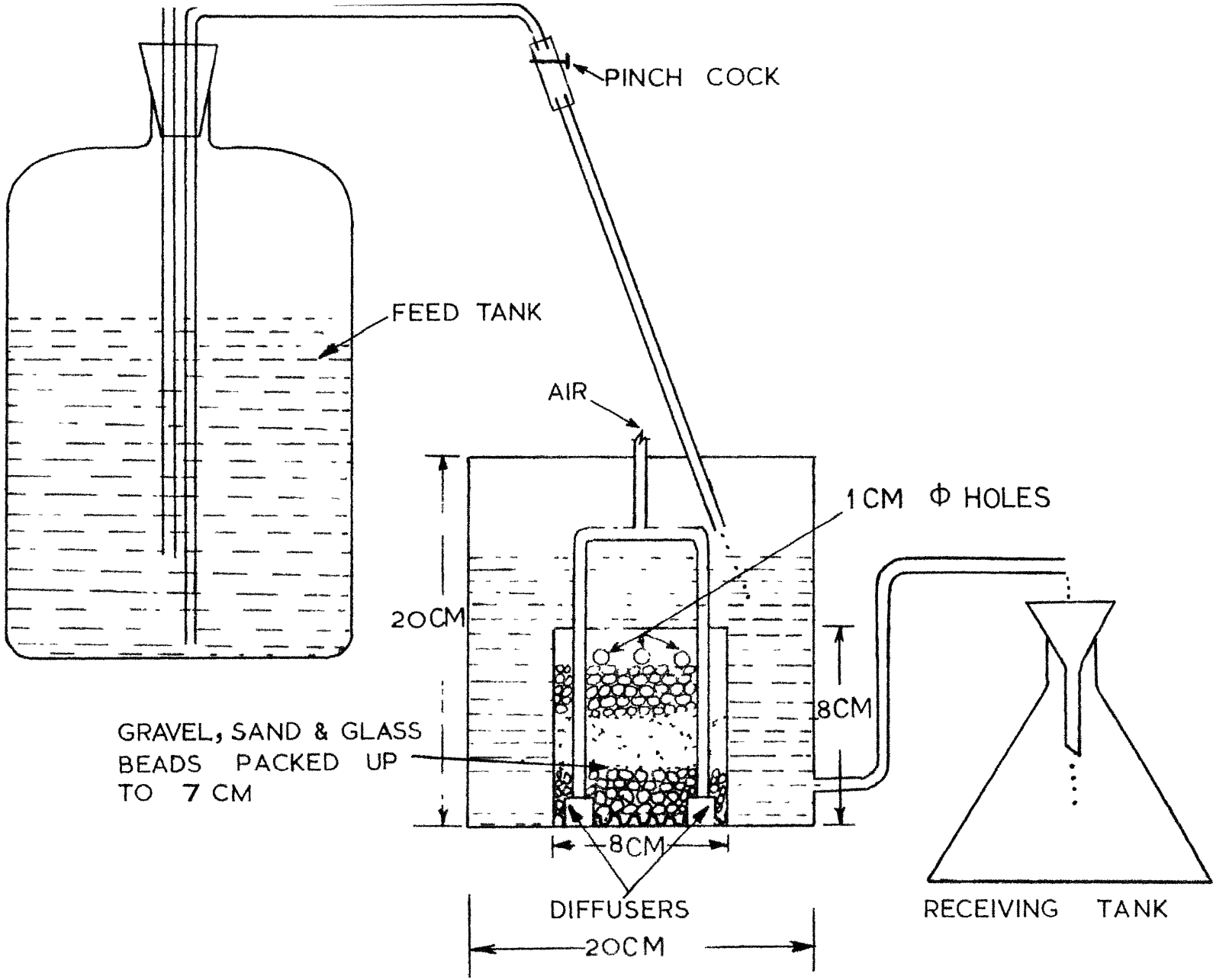


FIG 8 DETAILS OF CONTINUOUS FLOW REACTOR-VESSEL

a screw clamp fitted to the tube from the feed-tank and was held constant by using a constant head siphon (45), as shown in the Figure 8. The reactor-vessel was covered to prevent loss of evaporation.

Samples were collected every 8-hour interval and analyzed for NH_3-N, NO_2^--N, NO_3^--N, organic nitrogen, and pH. Determination of dissolved oxygen in the reactor was done from time to time to ensure aerobic conditions.

The study of nitrification in the continuous flow reactor was designed into two phases. The first phase was devoted to finding out design parameters involved, of hydraulic loading and maximum growth constant (μ_{max}) of the nitrifying bacteria. This was achieved by observing the rates of nitrification at various flow rates. The ammonia-nitrogen concentration in the feed was kept at 0.05 M and pH at 8.0. These values were selected on the basis of observations in the present study and earlier reports by various investigators.

The second phase was designed to find out the effect of substrate concentration. In this case, a flow rate of about 1 l/day, giving a F/V value of 8.33×10^{-3} hr^{-1} was adopted. This value was chosen on the basis of observations in the first phase. The pH was again maintained at 8.0 and only the concentration of ammonia-nitrogen in the feed-tank was varied.

In both phases of study, changes in operational parameters, flow rate and ammonia-nitrogen concentration in the feed, were changed only when the system had attained an equilibrium in terms of rate of nitrification.

6. Study of growth of nitrifying bacteria

This study was conducted to obtain growth kinetics of Nitrosomonas and Nitrobacter in batch systems and to obtain data to interpret and verify the observations made in the earlier phase of present study.

To obtain the growth curves, the microorganisms were cultured in two 1 l conical flasks, each containing a sand bed. The growth medium contained along with the minerals, 0.05 M NH_4Cl for Nitrosomonas and 0.05 M $NaNO_2$ for Nitrobacter. In both the cases pH was maintained at 8.0 by means of 0.1 M phosphate buffer and the flask contents were aerated by passing air through diffusers submerged in the sand bed.

While drawing samples for finding out MPN (Most Probable Number), the vessels were stirred along with the sand bed by means of a glass rod quite vigorously so that the sample might represent the true number of the nitrifying bacteria present in these vessels.

B. Analytical Techniques

1. Ammonia-Nitrogen

In the beginning, ammonia was estimated by the distillation method (i.e., Kjeldahl method) as given in the Standard Methods (46). Later on, however, for greater accuracy, ammonia was determined by direct nesslerization method. This was done with the help of a Spectronic 20 [1].

The standard calibration curve for NH_3-N is shown in Figure 9.

[1] Manufacturer : Bausch & Lomb Incorporated
Rochester, New York 14602

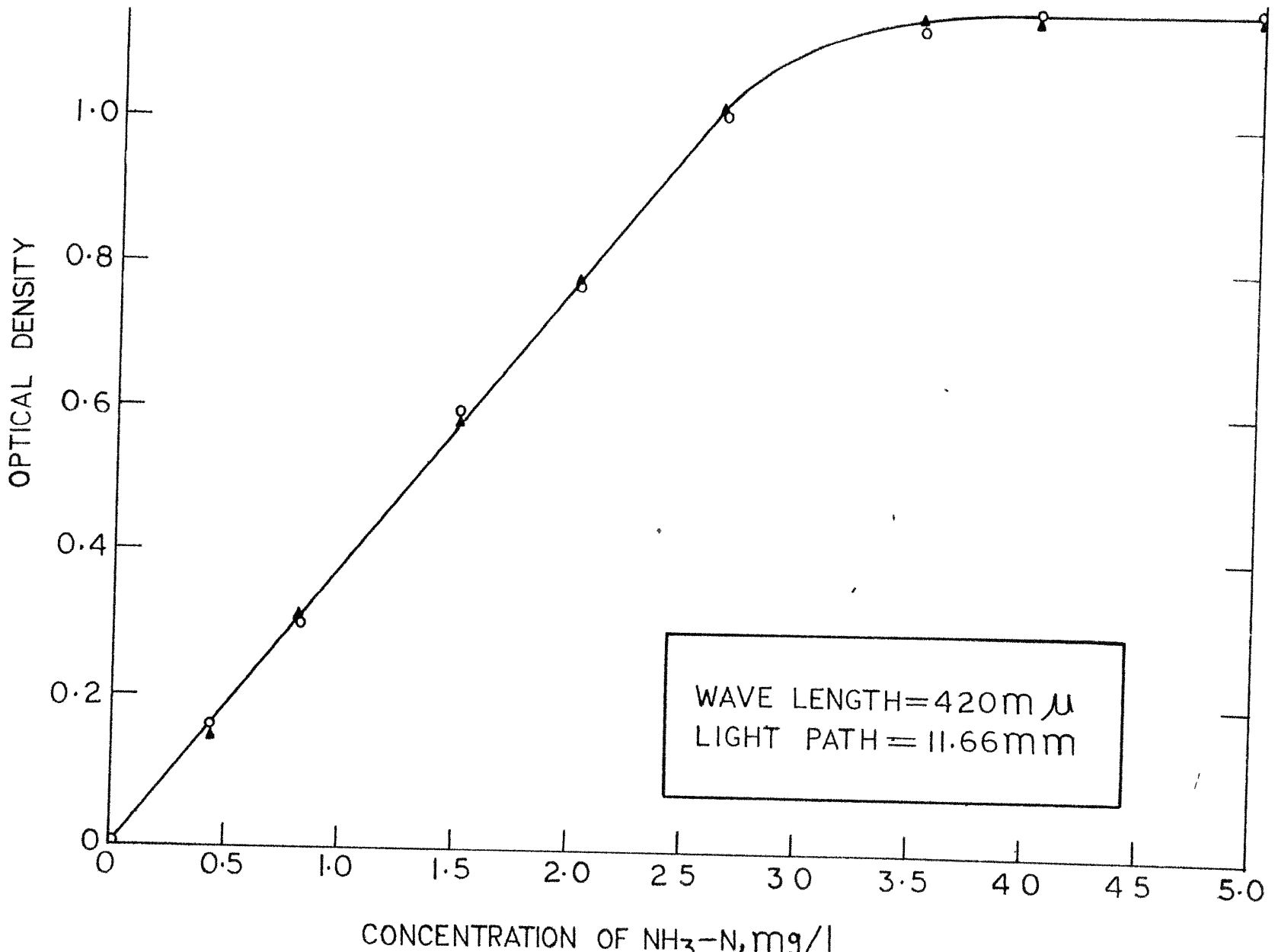


FIG 9 STANDARD CALIBRATION CURVE FOR NH_3–N, DIRECT NESSLERIZATION METHOD (46)

2. Nitrite-Nitrogen

Nitrite was determined by sulfanilic acid - naphthylamine hydrochloride method as suggested in the Standard Methods (46). In this case also, measurements were made by means of a Spectronic 20, discussed earlier.

The standard calibration curve for NO_2^--N is shown in Figure 10.

3. Nitrate-Nitrogen

Nitrate content of the samples was determined by Brucine method (46). As before, measurements were made by the Spectronic 20.

The standard calibration curve for NO_3^--N is shown in Figure 11.

4. Organic Nitrogen

Organic nitrogen was estimated by means of Kjeldahl method (46).

5. pH

pH of the samples was ascertained by means of an Expandomatic pH Meter [2]. For calibration of the pH meter, two standard buffers, one in the acidic range and the other in the alkaline range were used.

6. Dissolved Oxygen

Dissolved oxygen content of the samples was measured by means of a Precision Galvanic Cell Oxygen Analyser [3].

The standard calibration curve for the DO Probe is shown in Figure 12.

[2] Manufacturer : Beckman Instruments Inc.
Scientific and Process Instruments Division
Fullerton, California 92634

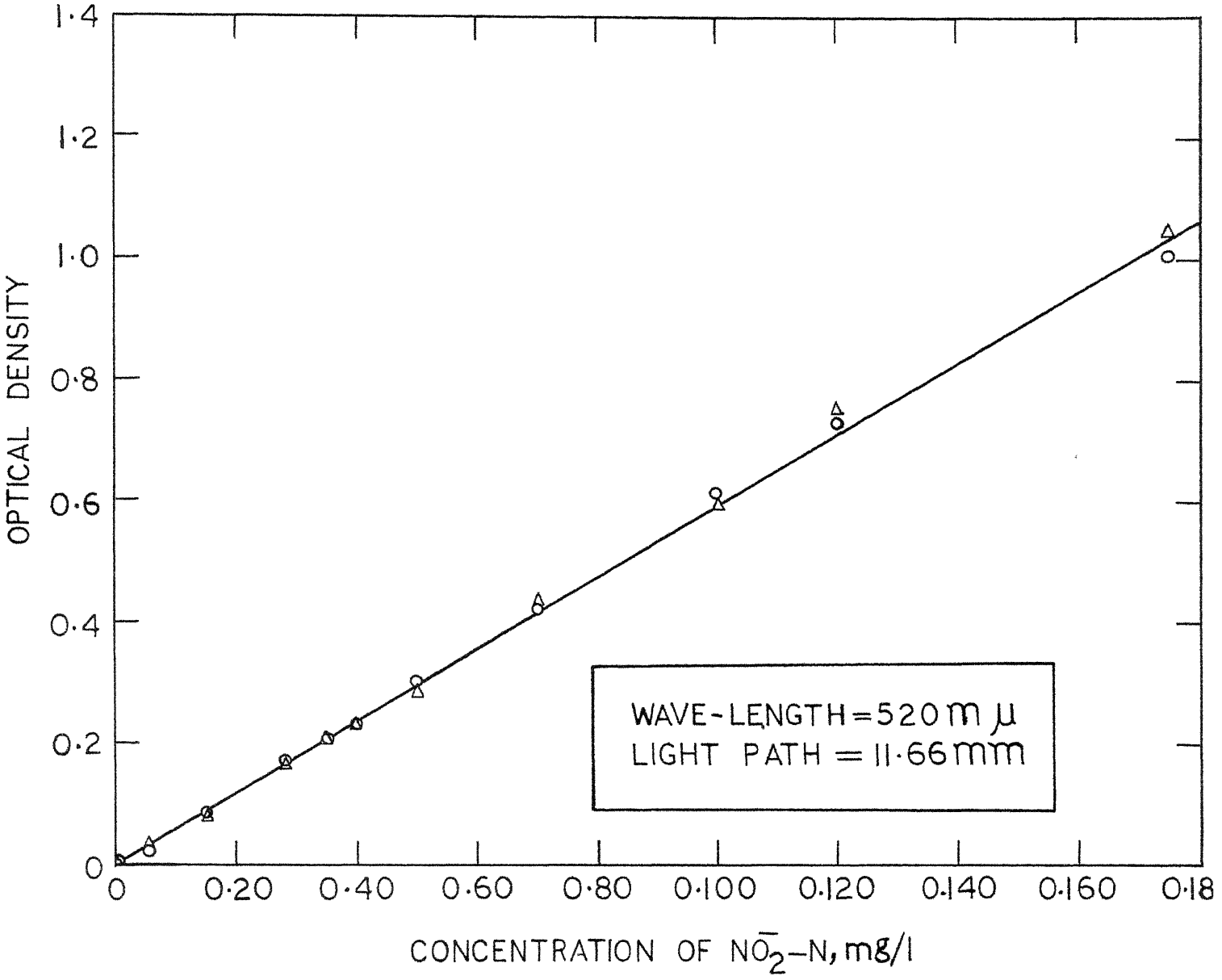


FIG.10. STANDARD CALIBRATION CURVE FOR NO_2^-–N, DIAZOTIZED SULFANILIC ACID METHOD (46)

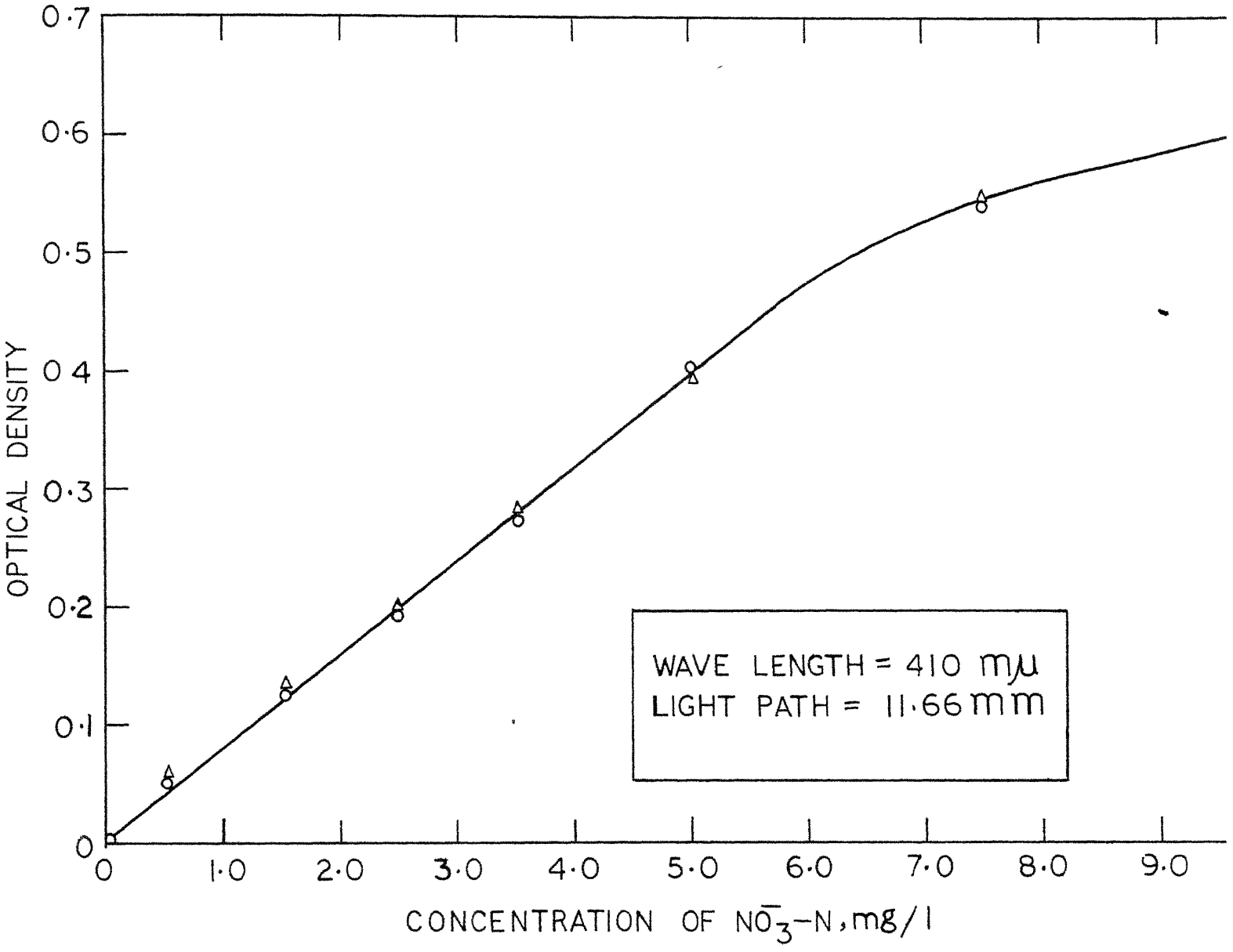


FIG 11 STANDARD CALIBRATION CURVE FOR NO_3^-–N, BRUCINE METHOD (46)

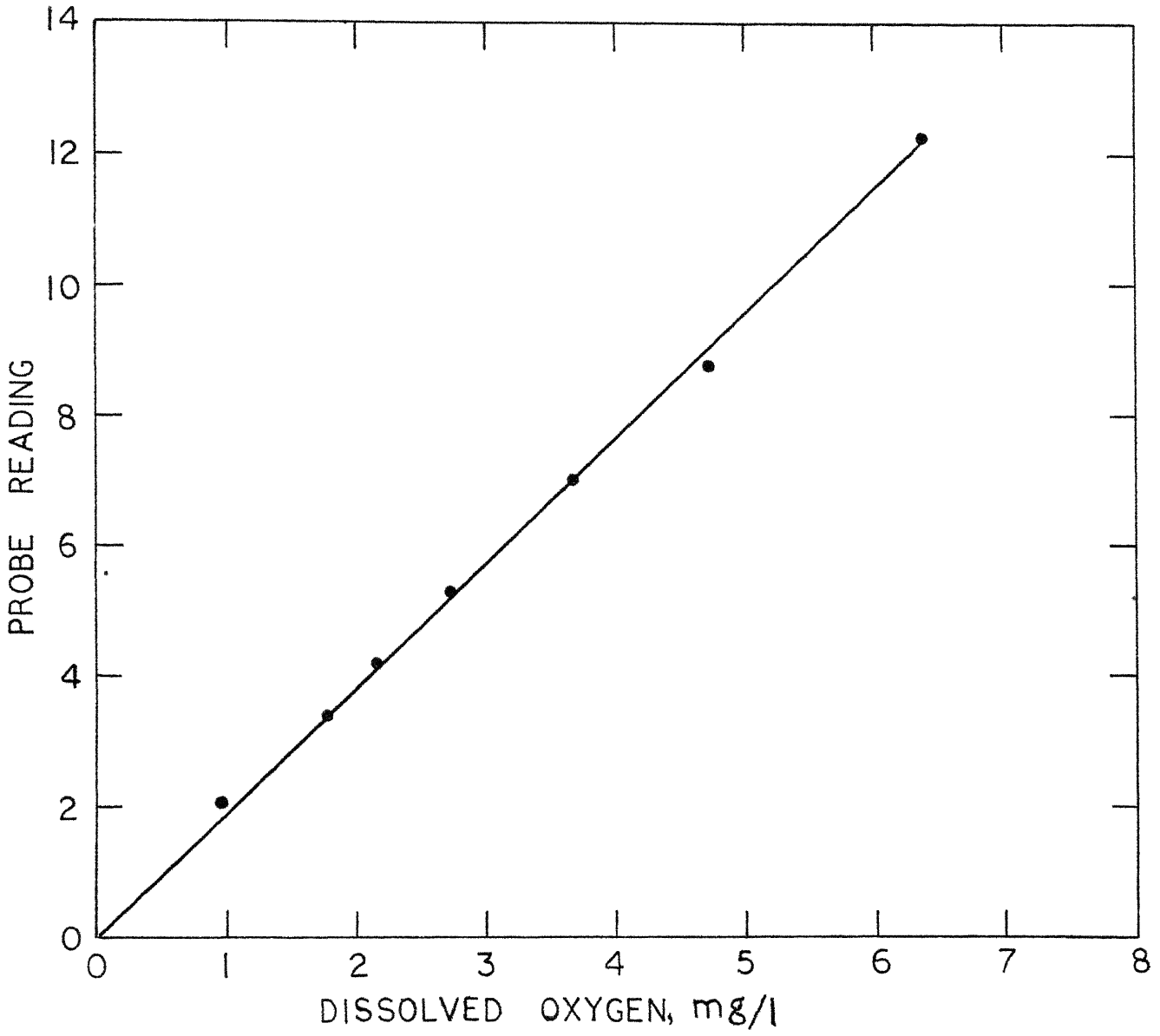


FIG.12.STANDARD CALIBRATION CURVE FOR DISSOLVED OXYGEN PROBE

7. Most Probable Number, MPN, of Nitrosomonas and Nitrobacter

Figure 13 represents the set-up for finding out the MPN of Nitrosomonas and Nitrobacter. The incoming air was saturated with water vapour by bubbling it through water in order to minimize evaporation losses from the culture tubes.

The culture tubes contained 0.05 M NH_4Cl solution for Nitrosomonas and 0.05 M $NaNO_2$ solution for Nitrobacter. Apart from these two specific substrates for the two types of nitrifying bacteria, each tube contained the same inorganic medium, discussed before. For each test, a set of 6 tubes, one contaning blank and 5 with samples were used. The total liquid volume in each tube was made up to 10 ml by adding distilled water. Each tube, excepting the two blanks, contained the nitrifying seed whose concentration was fixed by the expected number of nitrifying bacteria in a particular sample. pH in each tube was maintained at 8.0 by adding 0.1 M phosphate buffer.

The tubes containing 0.05 M NH_4Cl solution were tested for nitrite- and nitrate-nitrogen. Those tubes which had either of them were considered to be positive and the rest as negative. In a similar fashion, tubes containing 0.05 M $NaNO_2$ solution were tested for nitrate-nitrogen and whichever contained nitrate-nitrogen, was considered to be positive. These tests were done after the tubes were aerated for one day. In the trial runs, it was found that conversion of ammonia to nitrite by Nitrosomonas or conversion of nitrite to nitrate by Nitrobacter very well takes place after 24 hours and a longer period does not make a negative tube positive at all whatever be the extent of this period of aeration.

Computing and recording of MPN of Nitrosomonas and

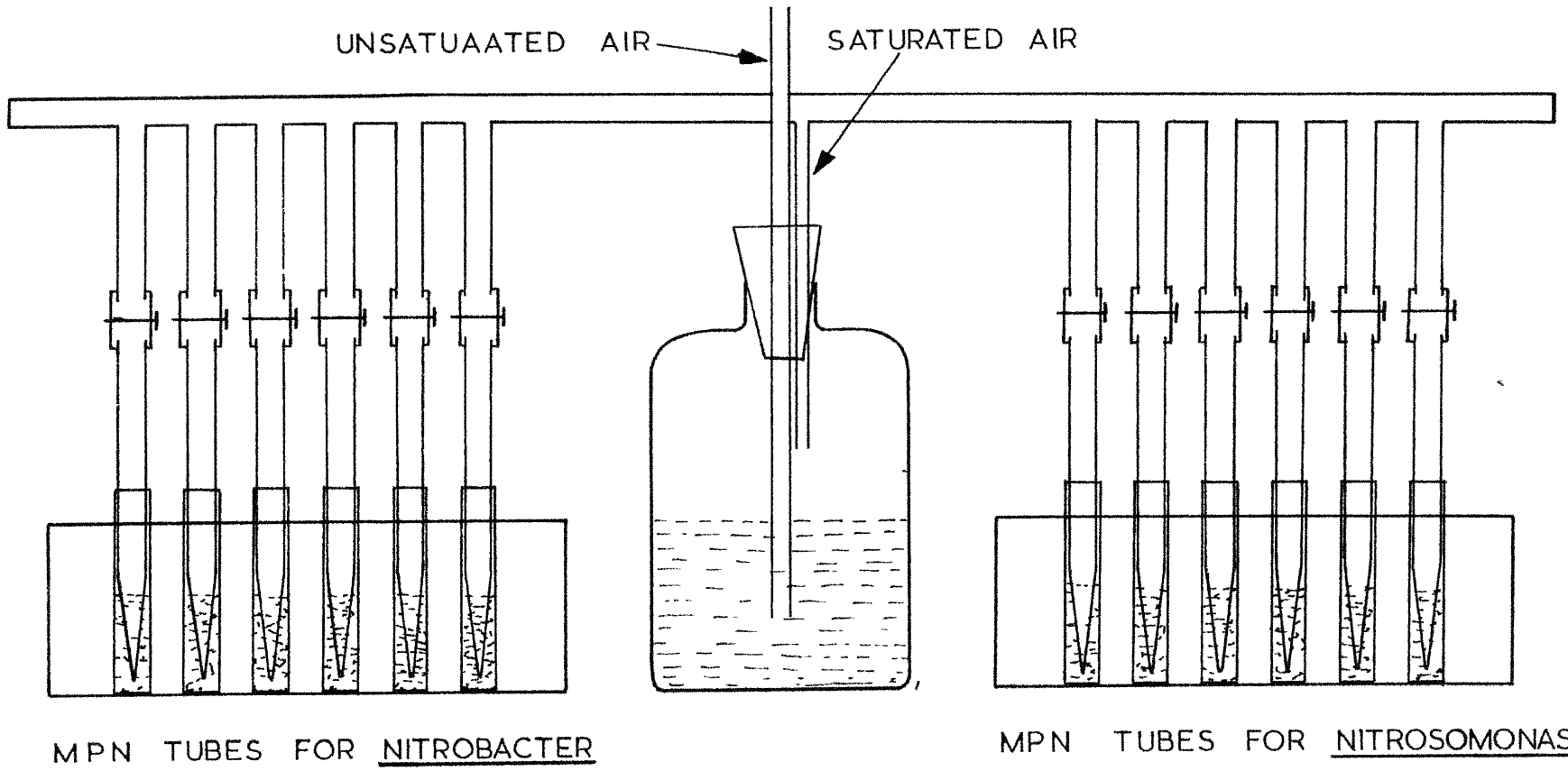


FIG.13 LABORATORY SET-UP FOR FINDING MPN OF NITROSOMONAS AND NITROBACTER

Nitrobacter at 95% confidence limits were made as per Standard Methods (46).

[3] Manufacturer : Precision Scientific Co.
3737 West Cortland Street
Chicago, Illinois 60647

V. RESULTS AND DISCUSSION

A. Inhibitory Concentration of Ammonia

To find out the inhibitory concentration of ammonia, several runs were carried out with different molar concentrations of NH_4Cl using the nitrifying column. Figures 14, 15 and 16 show the concentrations of ammonia-, nitrite-, and nitrate-nitrogen in the column with respect to time for different concentrations of feed. The sharp decrease in concentration of NO_2^--N and NO_3^--N and increase in NH_3-N resulted due to withdrawal of sample and addition of feed.

From Figure 14, it is seen that the second stage of nitrification i.e., oxidation of nitrite to nitrate is rather slow in the beginning. According to Fry (5), similar was the experience of Lees and Quastel who concluded that this is, perhaps, a lag period for Nitrobacter . For feed concentrations of 700 mg/l (0.05 M) and 1120 mg/l (0.08 M) of NH_3-N (Figures 14 and 15), nitrification proceeded satisfactorily and there were 70% and 85% conversions of NH_3-N during periods of 11 days and 18 days, respectively. Out of the total nitrogen nitrified, only 20% and 10% respectively were converted to nitrate-nitrogen.

From Figure 16, it is seen that when the feed contained 0.10 M NH_3-N, Nitrobacter bacteria were inhibited first as indicated by constant decrease in nitrate concentration every day due to withdrawal of samples and dilution by addition of feed containing NH_3-N only. This inhibition took place on the second day of addition of 0.10 M NH_3-N to the column. During the first day, the nitrite-nitrogen concentration in the column increased from 500 to 964 mg/l (0.069 M) and that of NH_3-N decreased from 1400 to 770 mg/l (0.055 M). Therefore, it may be concluded that the inhibition was brought about by a nitrite-nitrogen concentration of 964 mg/l (0.069 M).

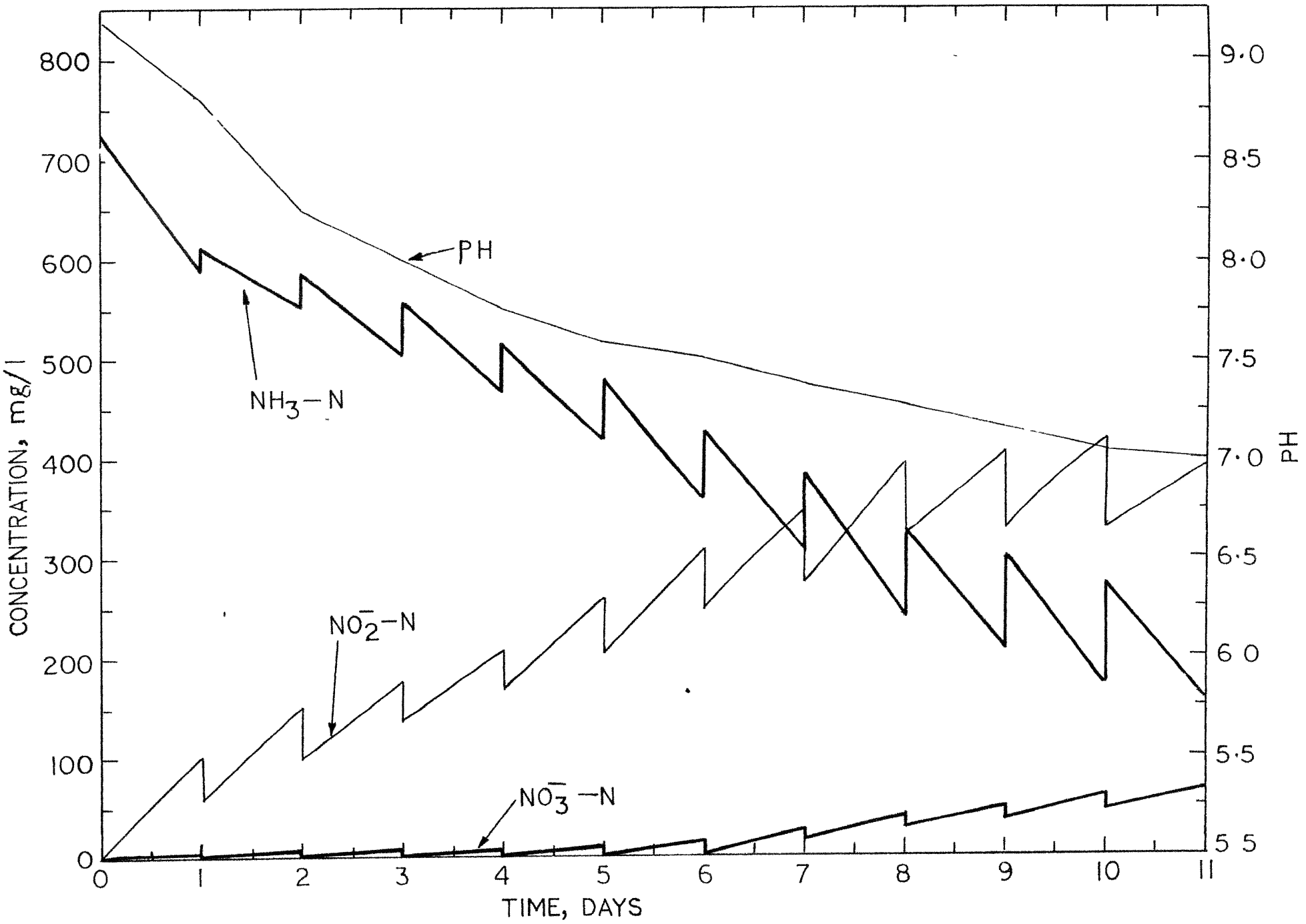


FIG 14 RATE OF NITRIFICATION AT AN INITIAL AND DAILY FEED CONCENTRATION OF 700 mg / l (0 05M) NH_3-N

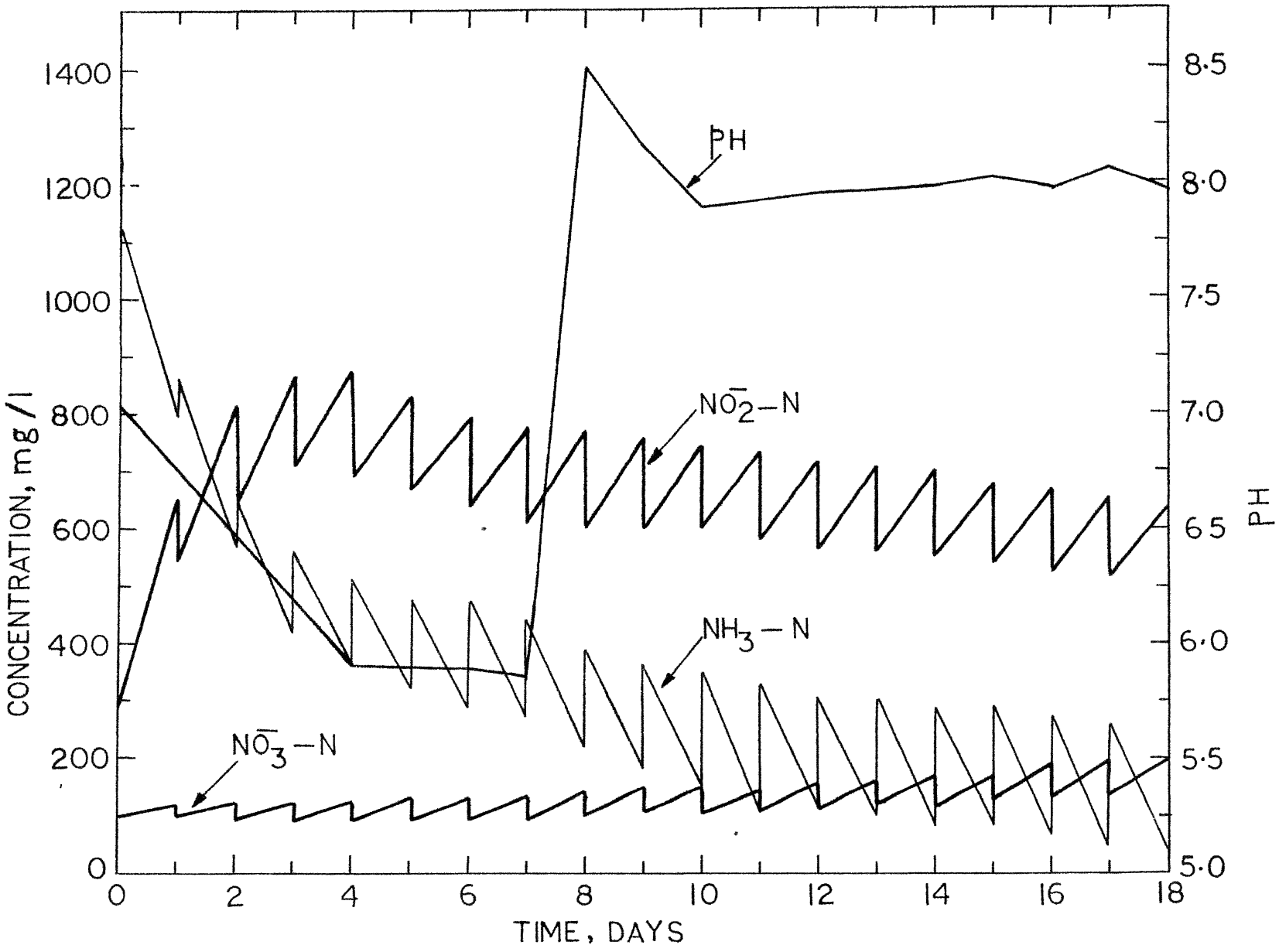


FIG.15. RATE OF NITRIFICATION AT AN INITIAL AND DAILY FEED CONCENTRATION OF 1120mg/l (0·08M) NH_3–N

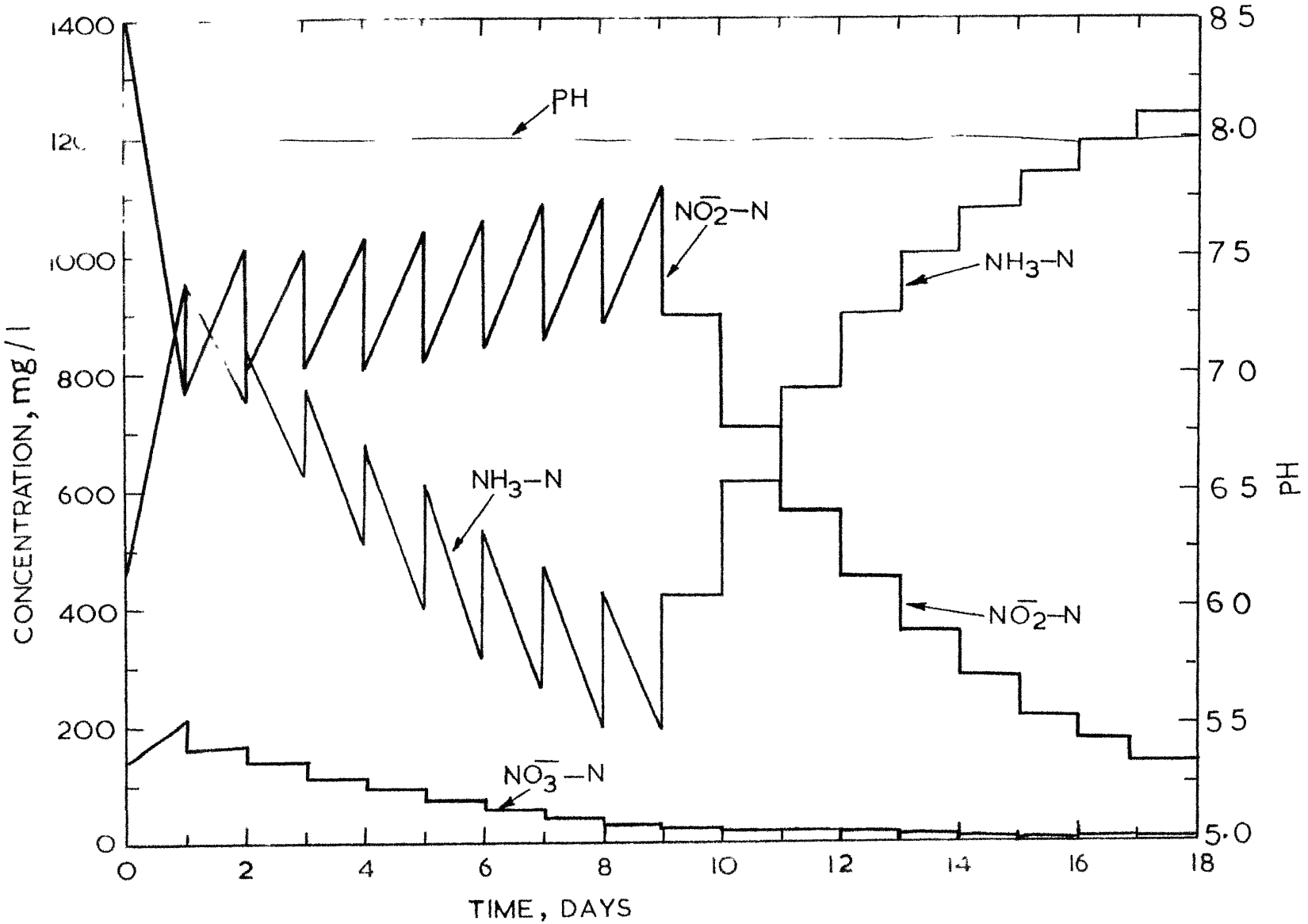


FIG 16. RATE OF NITRIFICATION AT AN INITIAL AND DAILY FEED CONCENTRATION OF 1400 mg/l (0·10 M) NH_3–N

While studying rate of nitrification by Nitrobacter agilis, Aleem and Alexander (31), and nitrification by a heterogenous population of nitrifying organisms, Meyerhof (34) concluded that nitrite accumulation becomes pronounced with greater ammonium salts applications which inhibit Nitrobacter.

It is further seen from Figure 16 that nitrification continued till the 9th day even though the ammonia-nitrogen concentration in the column was increased to 1400 mg/l (0.10 M) initially. During this period, the nitrite-nitrogen concentration reached a level of 1110 mg/l. But from the 9th day, nitrite-nitrogen concentration started decreasing showing that the Nitrosomonas were inhibited. This indicates that the inhibition of Nitrosomonas was due to 1110 mg/l (0.0799 M) of NO_2^--N. This observation being contradictory to the reports that it is 0.10 M NH_3-N which inhibits Nitrosomonas, another set of experiments, described below, were conducted for verification.

Extent of nitrification by a nitrifying seed was observed in three series of medium tubes having different concentrations of NH_3-N and NO_2^--N as shown in Table 8. Table 8 also shows the extent of nitrification after 24 hours. These results are summarized in Figure 17.

It is seen that in the case of Series I, 0.10 M NH_3-N does inhibit Nitrosomonas as in tubes containing 0.10M and higher (0.12 M) concentrations of NH_3-N, there was no appreciable nitrification. The observation in the experiment using column that 0.10 M NH_3-N was converted to NO_2^--N by Nitrosomonas may be because there were some tolerant microorganisms which could sustain the dose and brought down the NH_3-N concentration to a level at which other organisms could

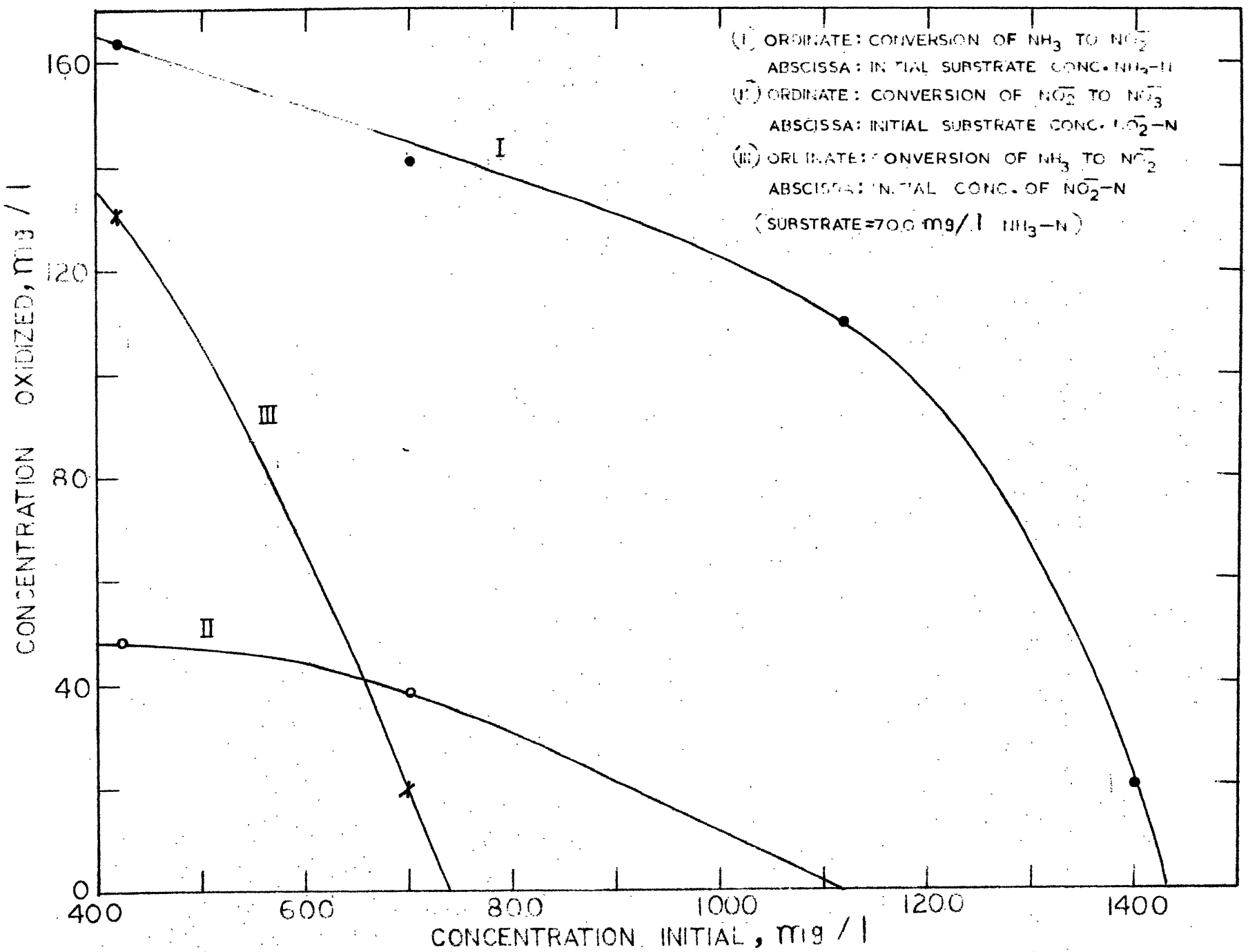


FIG-17. INHIBITORY CONCENTRATION OF NH_3-N AND NO_2^--N FOR NITROSOMONAS AND NITROBACTER

TABLE 8

NITRIFICATION IN 24 HOURS AT VARIOUS SUBSTRATE CONCENTRATIONS FOR A CONSTANT INOCULUM OF NITRIFIERS

SERIES I	Initial	NH_3-N, mg/l	420 (0.03 M)	700 (0.05 M)	1120 (0.08 M)	1400 (0.10 M)	1680 (0.12 M)
	Final	NO_2^--N, mg/l	126	112	79	16.2	0
		NO_3^--N, mg/l	38	39.4	30.4	3.64	0
SERIES II	Initial	NO_2^--N, mg/l	420 (0.03 M)	700 (0.05 M)	1120 (0.08 M)	1120 (0.08 M)	1400 (0.10 M)
	Final	NO_3^--N, mg/l	48	38	0	0	0
SERIES III	Initial	NH_3-N, mg/l	700 (0.05 M)	700 (0.05 M)	700 (0.05 M)	700 (0.05 M)	700 (0.05 M)
		NO_2^--N, mg/l	420 (0.03 M)	700 (0.05 M)	1120 (0.08 M)	1120 (0.08 M)	1400 (0.10 M)
	Final	NO_2^--N, mg/l	519	680	1116	1118	0
		NO_3^--N, mg/l	32.4	41	0	0	0

oxidize it. However, the above idea is questionable till the presence of a tolerant strain is demonstrated.

The conclusion from observations of Series II that 1120 mg/l NO_2^--N inhibits Nitrobacter further support earlier observation. For Series III in which varying concentrations of NO_2^--N with an optimum concentration of NH_3-N (700 mg/l) were added, it is seen from Figure 17 that oxidation of NH_3-N would stop when nitrite-nitrogen concentration is 750 mg/l. This observation supports the result obtained in the study using the nitrifying column.

The mass-balance for nitrogen showed a fairly good equality, discrepancy being to the extent of 5 to 7 mg/l. Out of this, some can be accounted for as the cellular requirement of the organisms. A few organic nitrogen determinations carried out during this study indicated an organic nitrogen concentration of 4 to 5 mg/l. Furthermore, Hofman and Lees (36) concluded that the discrepancy in such a mass-balance may be due to the formation of some intermediate product. Their studies were correlated with the theory of Kluyver and Donker (36) that the oxidation of ammonia by Nitrosomonas proceeds via hydroxylamine according to the following step :

$$NH_3 \xrightarrow{A} NH_2OH - (H_2N_2O_2) \xrightarrow{B} HNO_2 \qquad (19)$$

B. Effect of pH

The effect of pH on the activities of nitrifying bacteria was studied in two series of experiment using the nitrifying column. In the first one , pH was veried from 8.0 to 9.5 and in the second one, from 8.0 to 5.5. Figures 18 and 19 show the concentrations of nitrite and nitrate in the sample drawn each day from the nitrifying column.

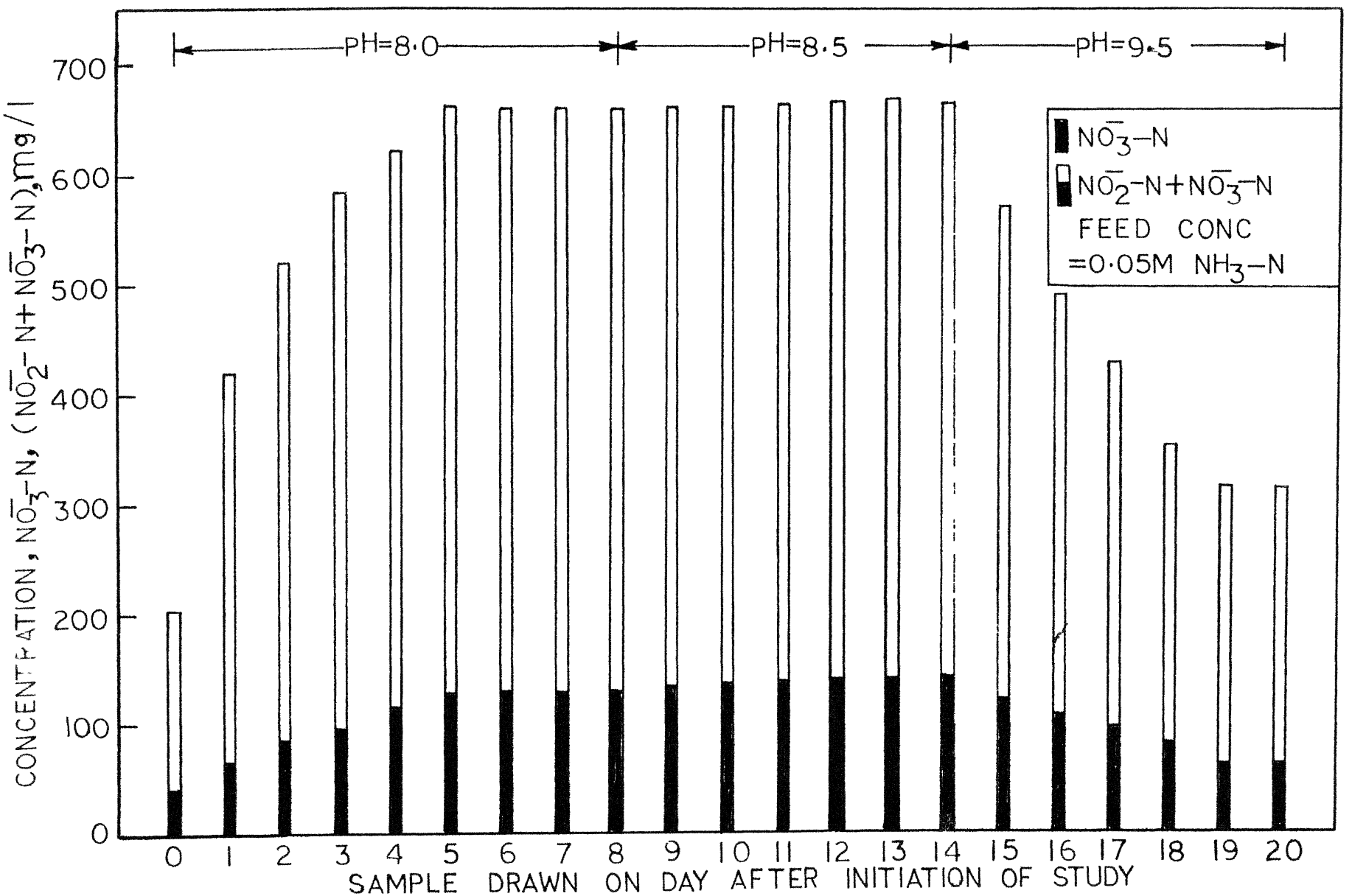
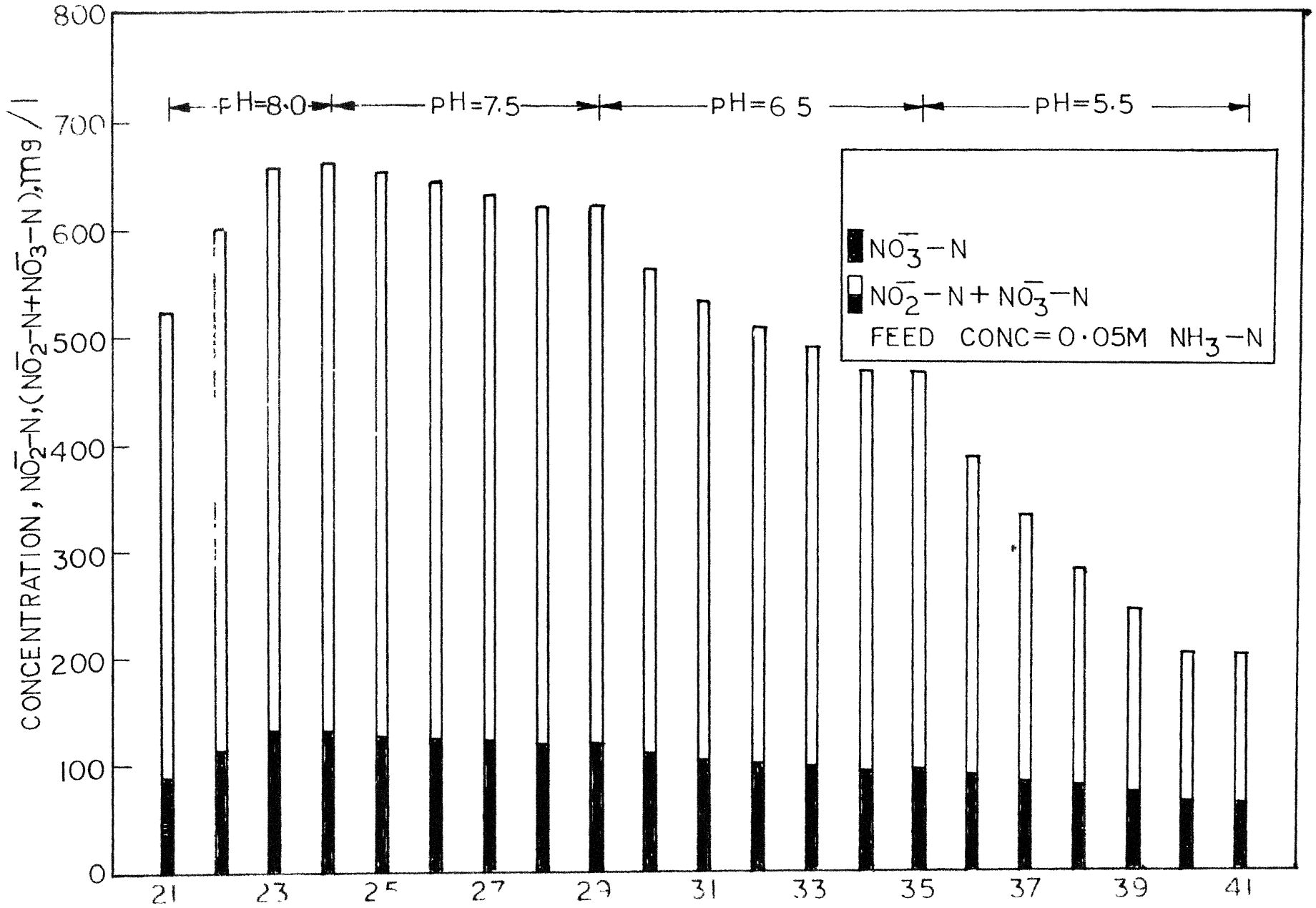


FIG 18 EFFECT OF pH ON NITRIFICATION, pH 8·0–9·5



FIG·19·EFFECT OF pH ON NITRIFICATION, pH 8 0–5·5

change from one pH level to another was made only after the samples showed constant concentrations of nitrite- and nitrate-nitrogen for at least two days. Percent of maximum rate of nitrification calculated from these values is shown in Figure 20. The curve for nitrite-nitrogen includes the quantity of nitrite-nitrogen which was converted to nitrate-nitrogen.

It is seen that the optimum pH level for Nitrosomonas lies between 7.8 and 8.3 whereas the optimum pH range for Nitrobacter is between 8.0 and 8.5. Meyerhof (34) (47), however, found that the optimum pH for the oxidation of ammonia (i.e., for Nitrosomonas) lies between 8.5 and 8.8 and that for nitrite oxidation (i.e., for Nitrobacter), between 8.3 and 9.3. The steep decrease in the acid side as found by Meyerhof (47) was not confirmed by Hofman and Lees (36). They found a much gradual fall, reaching a little above 50% of the maximum rate at pH 6.5. Figure 20 shows a fairly good conformity to these results.

For Nitrobacter, Hofman and Lees (36) reported the tendency of the curve somewhat more to the acid side as compared to that of Nitrosomonas, the optimum pH for Nitrobacter being 7.7 while Meyerhof (34) reported the optimum pH for Nitrobacter to be between 8.3 and 9.3. The optimum pH found in the present study for Nitrobacter lies between 8.0 and 8.5 which is closer to Meyerhof's results.

According to Aleem and Alexander (31), Nitrobacter agilis can respire within a wide pH range, 5.5 to 9.8. They found that greatest oxygen uptake rate was at pH 8.0 although the respiratory rate of this organism was quite high at pH 7.0 and 8.5. The latter part of their results very well fits with the results shown in Figure 20 which shows 95% rate of formation of nitrite-nitrogen between pH 7.5 and 8.6, and

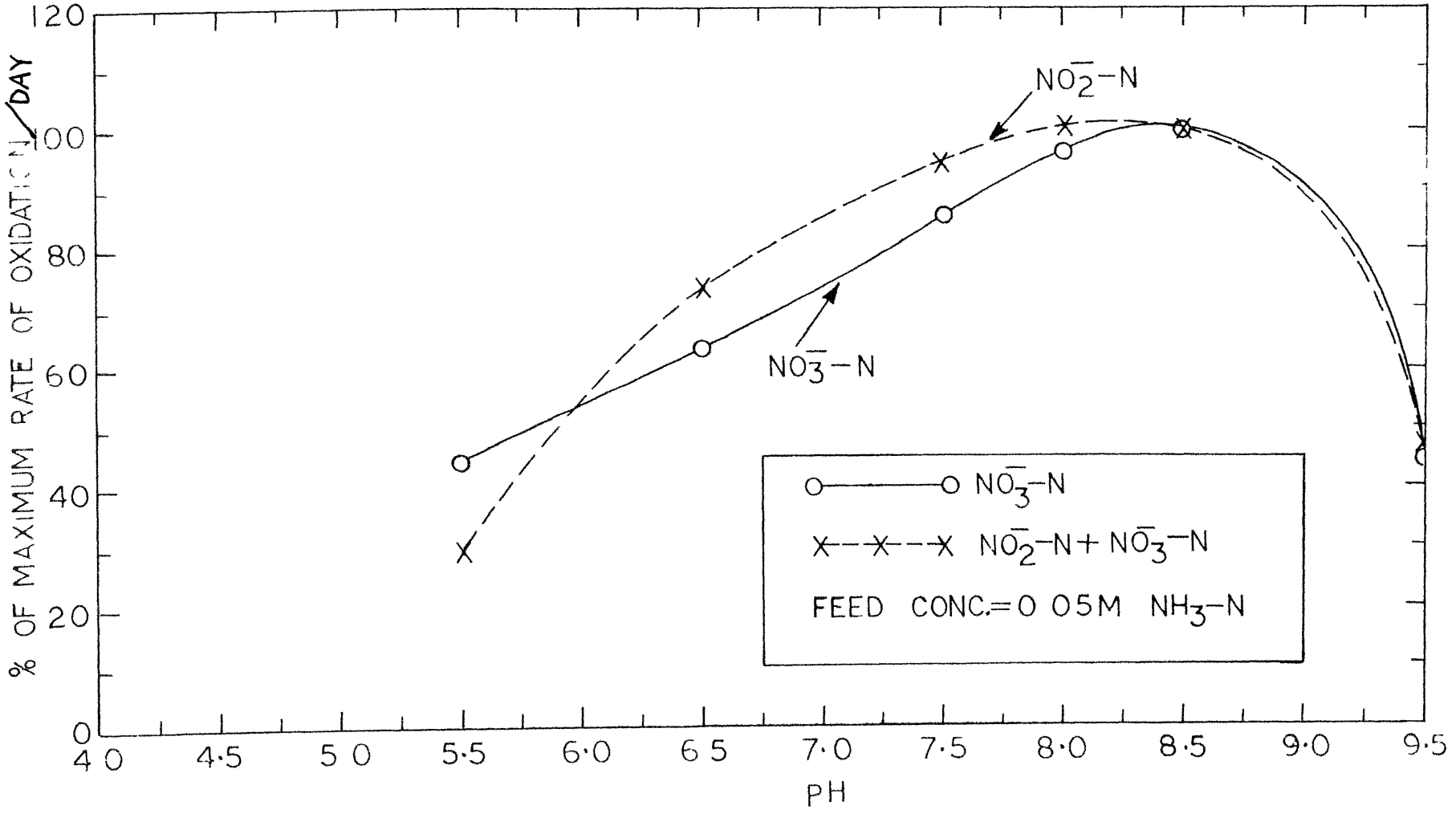


FIG.20. EFFECT OF PH ON RATE OF NITRIFICATION

75% rate of formation of nitrate-nitrogen between pH 7.0 and 9.2. Unlike Meyerhof's (34) results, it is seen from Figure 20 that Nitrosomonas are active even at an acidic condition of pH 5.5 or at an extremely high alkaline condition of pH 9.5. The same statement holds good for Nitrobacter and, in this case, the fall in the rate of nitrification is more gradual than that in the earlier case. Stephenson (47) pointed out that organisms isolated from peat soils (pH 4.6) display a greater tolerance for acid, nitrification continuing at as low a value as pH 4.1.

According to Stephenson (47), the high optimum alkalinity and the rapid falling off in activity on both sides of the optimum value may be due to secondary inhibitory effects which become operative on both acid and alkaline sides. Probably as Meyerhof (47) suggested, the rapid drop on the alkaline side is due to the penetration of the cell by free ammonia. It was shown both by Warington (38) and by Winogradskyi (47) and confirmed by Meyerhof (47) that the oxidation of nitrite is also unfavourably affected by ammonium salts. However, the narrow pH optimum with a steeper fall on the alkaline side than on the acid side in this study favours Thimann's (34) view that the cells may be unable to use the carbonate ion as a source of carbon dioxide while they could use bicarbonate ion on the alkaline side.

However, from the studies of Meyerhof (34) it is not clear whether the cells were acclimated before the rates of nitrification were calculated. In the present study, the organisms had sufficient opportunity for acclimation as the experiments were conducted only after a substantial growth of the nitrifying bacteria as indicated by appreciable rate of nitrification was noted.

C. Nitrification in a Completely Mixed Continuous Flow Reactor

1. Reactor design

The two types of biological reactor used in Sanitary Engineering practice are exemplified by anaerobic digesters and trickling filters. In the latter, a supporting medium for the growth of microorganisms is provided while in the former the microorganisms remain in suspension. Before initiating the present phase of the study, both these two types were tested for their suitability.

The reactor used has been described in the last chapter. First, it was started in absence of the sand bed. Figure 21 depicts the rate of nitrification without the sand bed till the 13th day, after which the sand bed was placed inside and subsequent rate of nitrification is also shown in Figure 21. It is seen that the rate of nitrification improved considerably by providing the sand bed. During this study, the reactor was filled once at the start with 140 mg/l (0.01 M) NH_3-N. The pH was maintained at 8.0.

Next the reactor was fed continuously after removing the sand bed, the feed concentration being maintained at 700 mg/l (0.05 M). The flow-rate was kept at 2160 ml/day and the pH was maintained at 8.0. From Figure 22, it is seen that this experiment resulted in a complete wash-out. This wash-out, it may be apprehended, was due to high flow-rate. But in studies described later, with the provision of the sand bed, it has been found that nitrification continues at this flow-rate. Therefore, it is presumed that the sand bed promotes the growth of microorganisms and in its absence, a high flow-rate in excess maximum growth rate constant, μ_{max}, may be responsible for a complete wash-out. In Figure 22, it is seen that the theoretical curve drawn with the assumption of no growth of nitrifying bacteria, closely proximates the curve plotted with observed values. It is,

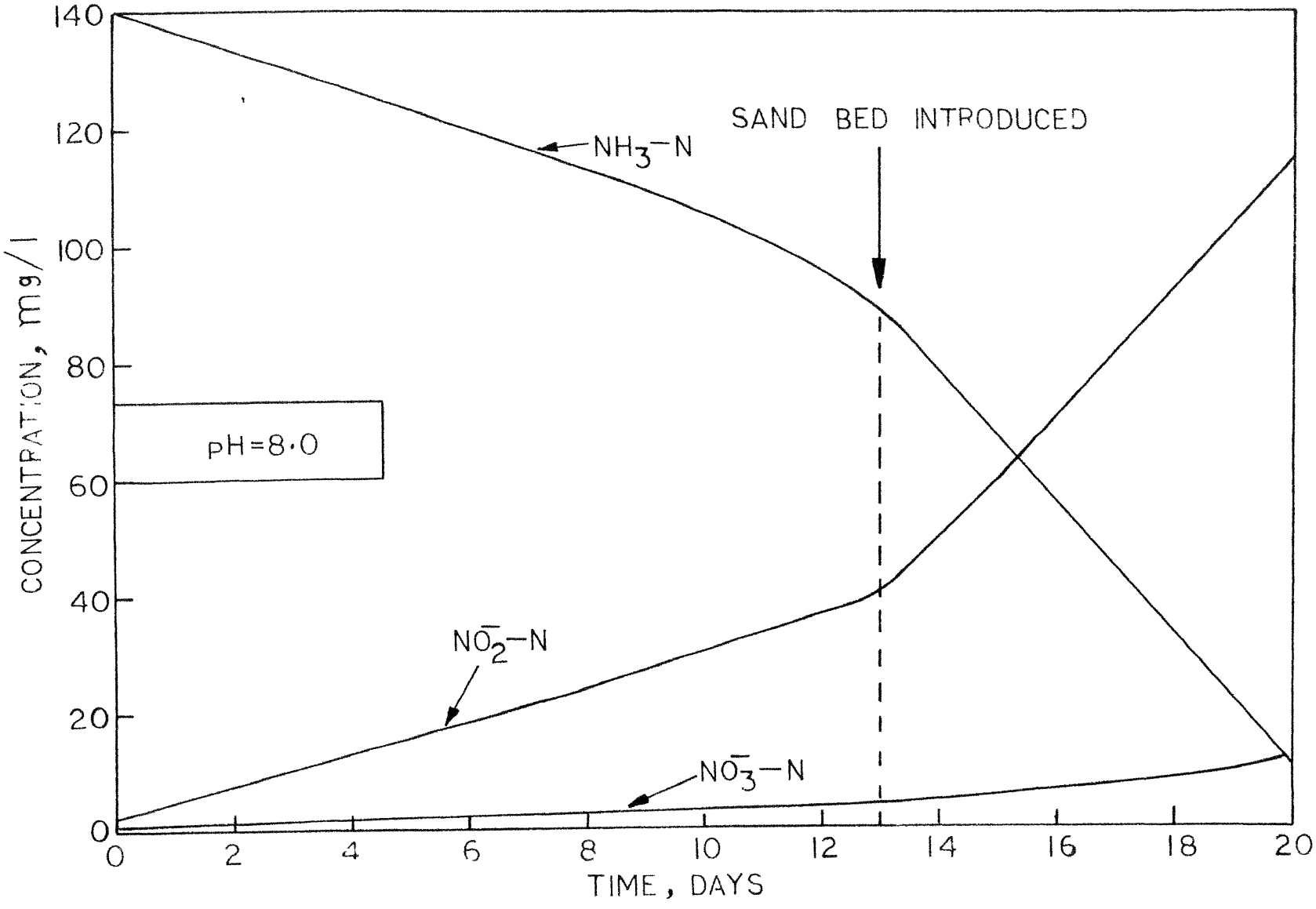


FIG 21 NITRIFICATION WITH AND WITHOUT SAND BED

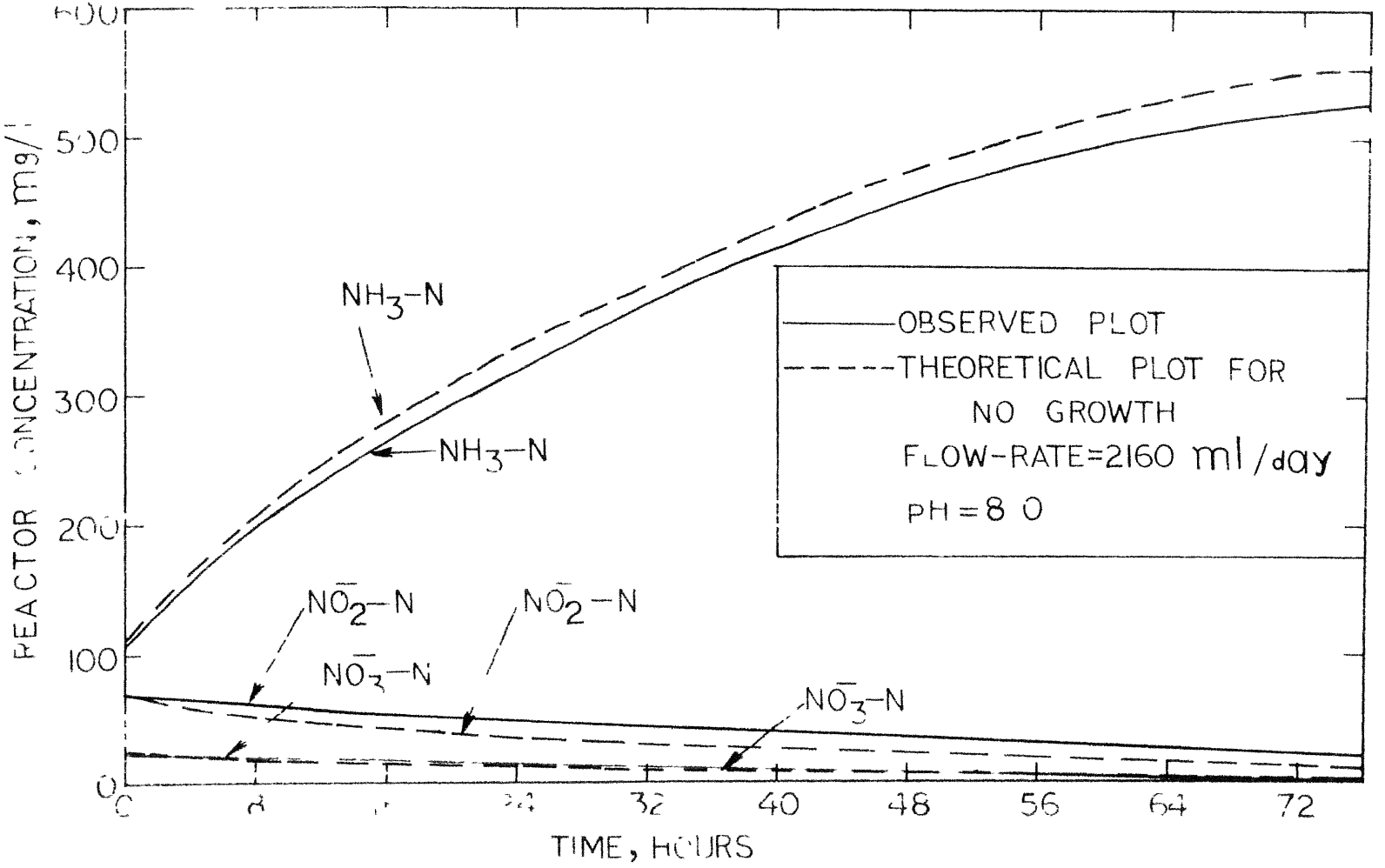


FIG 2 2. THEORETICAL AND OBSERVED PLOTS FOR WASH OUT

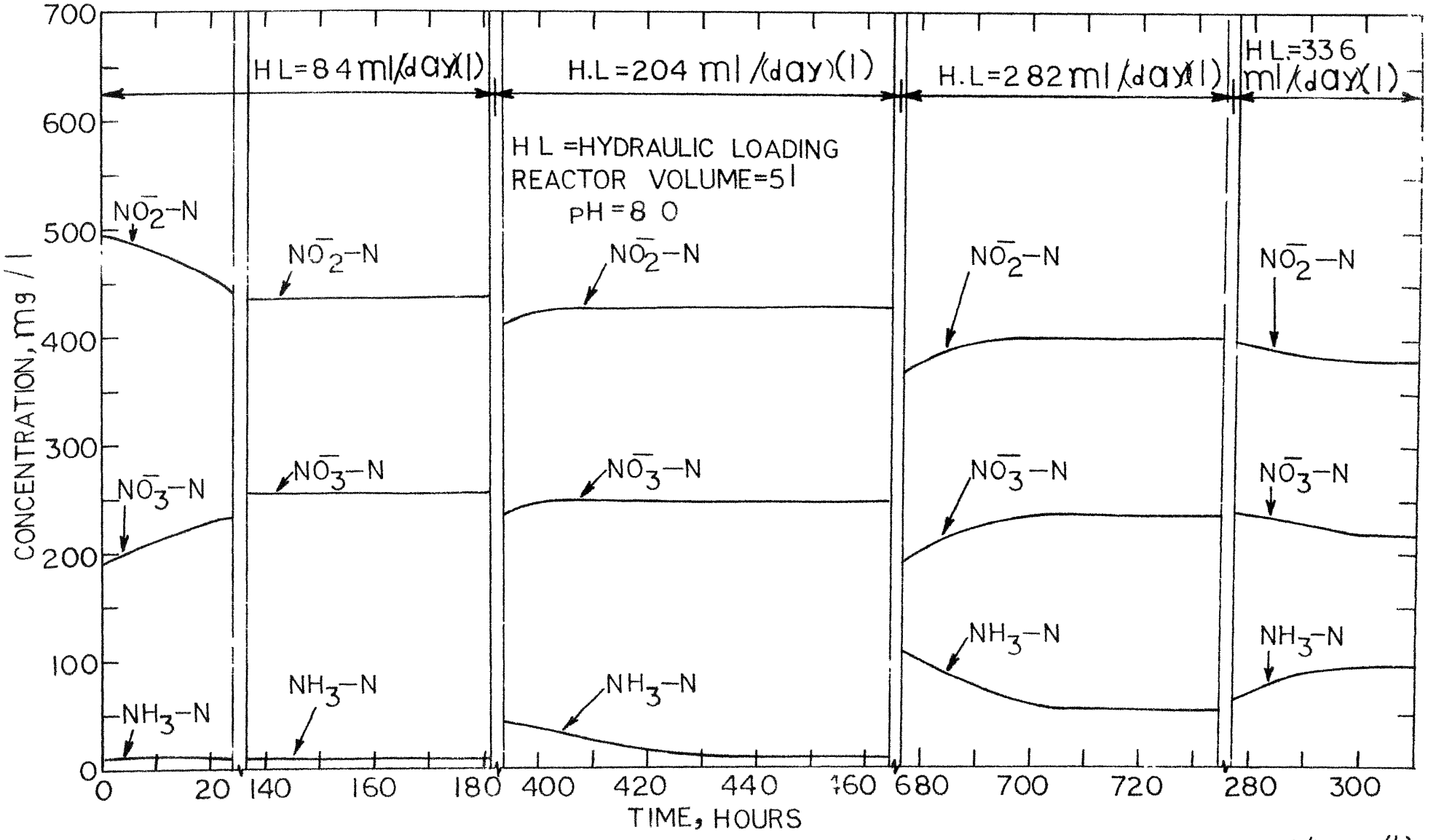


FIG.23. NITRIFICATION WITH RESPECT TO HYDRAULIC LOADING VARYING FROM 84 TO 336 ml/(day)(l)

therefore, concluded that no significant nitrification occurred at a flow-rate of 2160 ml/day in the absence of the sand bed and ultimately it resulted in complete wash-out.

Therefore, all the experiments carried out later were designed with the provision of a sand bed to ensure high rate of nitrification.

2. Effect of hydraulic loading

In this phase of study, a feed concentration of 700 mg/l (0.05 M) NH_3-N, as in the case of studying the effect of pH on nitrification, has been chosen because it has been found from earlier results that at this particular concentration of NH_3-N, the extent of nitrification is quite high and, moreover, this may relate to actual influent concentration of NH_3-N at various fertilizer plants. For all these runs, pH was maintained at 8.0.

Figures 23 and 24 show how nitrification proceeded with respect to time when flow-rates and, therefore, hydraulic loadings expressed in ml/(day)(l) were varied. Since it was not known at which hydraulic loading wash-out would occur, increasing order of hydraulic loadings as shown in Figures 23 and 24 were not tried in time sequence. This is apparent from the abscissa of these two plots.

It is seen from Figure 23 that almost complete nitrification occurred up to a hydraulic loading of 204 ml/(day)(l). The effluent contained about 61% NO_2^--N and 34% NO_3^--N of the NH_3-N concentration in the feed. Increasing the hydraulic loading above 204 ml/(day)(l) resulted in incomplete nitrification. At a hydraulic loading of 384 ml/(day)(l), total nitrification was only to the extent of 50%. Such a hydraulic loading for an influent NH_3-N concentration of 700 mg/l would, therefore, not be suitable if complete nitrification is required.

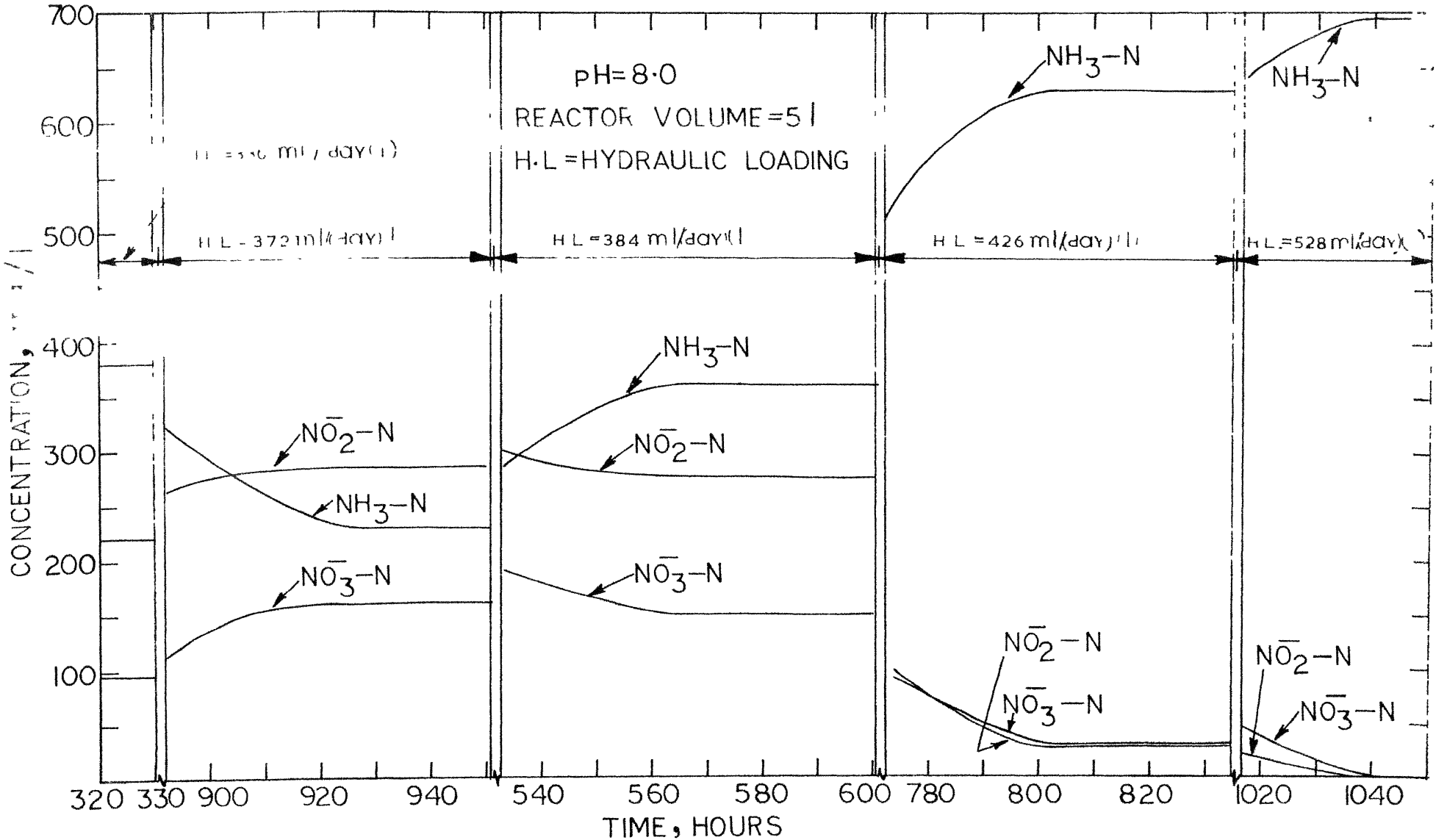


FIG 2 4 NITRIFICATION WITH RESPECT TO HYDRAULIC LOADING VARYING FROM 336 TO 528 ml /(day)(l)

In order to relate the above data to growth characteristics of microorganisms and thereby develop design criteria, they are plotted in Figure 25 in accordance with Figure 5 given in chapter II.

The curves in Figure 25 have been plotted by considering concentrations of NH_3-N, NO_2^--N, and NO_3^--N at equilibrium conditions of different hydraulic loadings versus the dilution rate, D. From this graph, it is seen that as the dilution rate increases above 25×10^{-2} day^{-1} there is an appreciable concentration of NH_3-N in the effluent.

In this context it may be noted that during denitrification, NO_3^--N is converted to NO_2^--N and thereafter, further reduction takes place. Since the proposed treatment process comprises of both nitrification and denitrification, nitrification may be continued only up to the oxidation of NH_3-N to NO_2^--N and further oxidation of NO_2^--N to NO_3^--N is not necessary. Hence in the present study, formation of NO_2^--N only has been emphasized upon. However, while considering the formation of actual NO_2^--N as a result of oxidation of NH_3-N, total of both NO_2^--N and NO_3^--N formed has been taken into account.

It may be noted that in this case, the mass of nitrifying bacteria found in the effluent would not represent the total number of them actually present in the reactor. It is quite likely that some of these bacteria would be sticking to the sand bed which provides them with a support. Furthermore, it may be assumed that only those bacteria which are sticking to the sand bed, X_1, are capable of multiplication while those nitrifying bacteria which are in suspension and are coming out in the effluent, X_2, cannot multiply and, therefore, do not contribute to nitrification.

The steady-state relationships for concentrations of microorganisms,

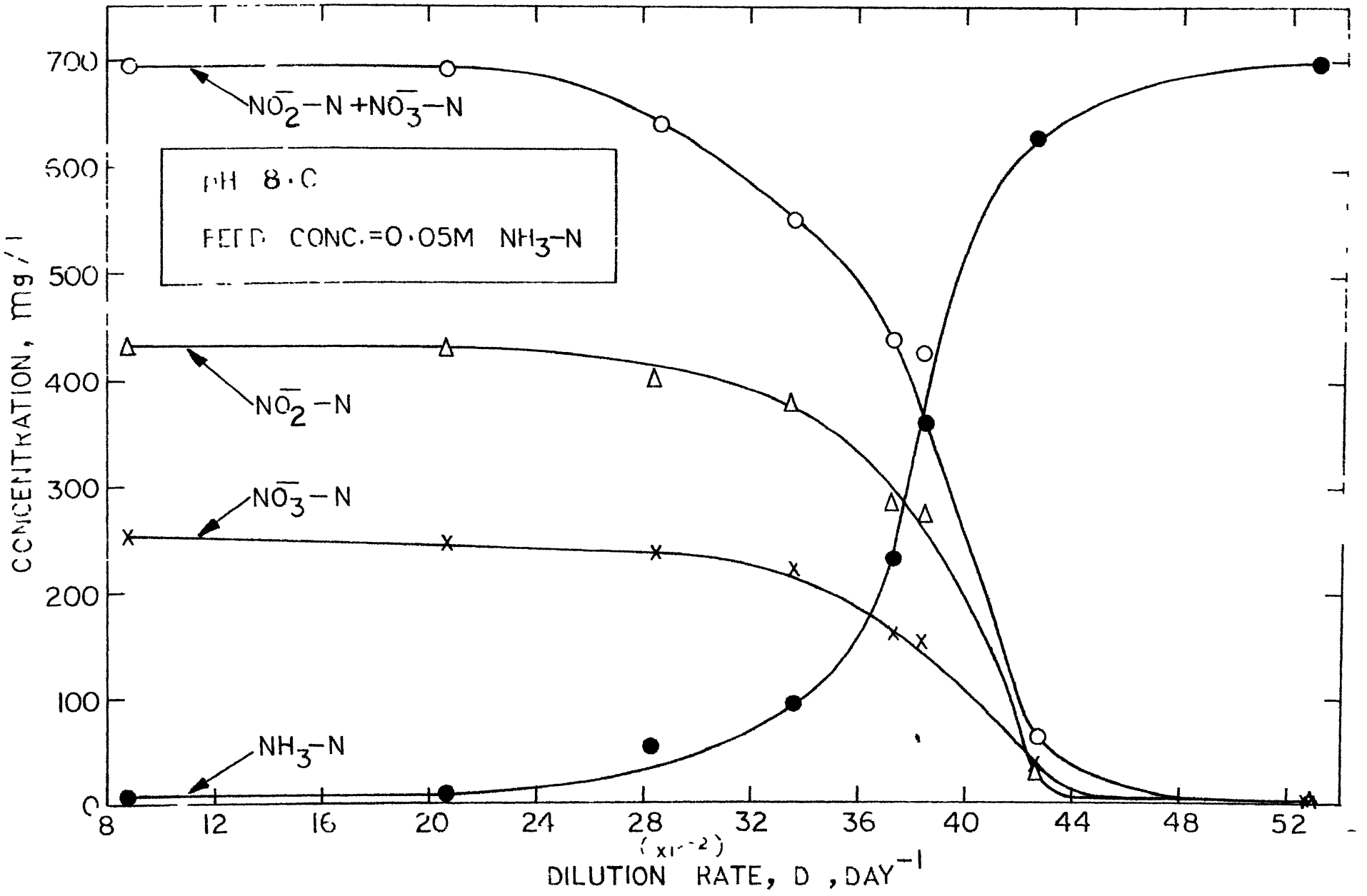


FIG 25 RATE OF NITRIFICATION IN CONTINUOUS FLOW REACTOR

product and substrate for the laboratory reactor can be written, respectively, as:

$$\frac{dX_1}{dt} = \mu X_1 - DX_2 = 0 \qquad (20)$$

$$\frac{dP}{dt} = Y_{1/X} \mu X_1 - DP = 0 \qquad (21)$$

$$\frac{dS}{dt} = D(S_o - S) - \frac{1}{Y_{X/S}} \mu X_1 = 0 \qquad (22)$$

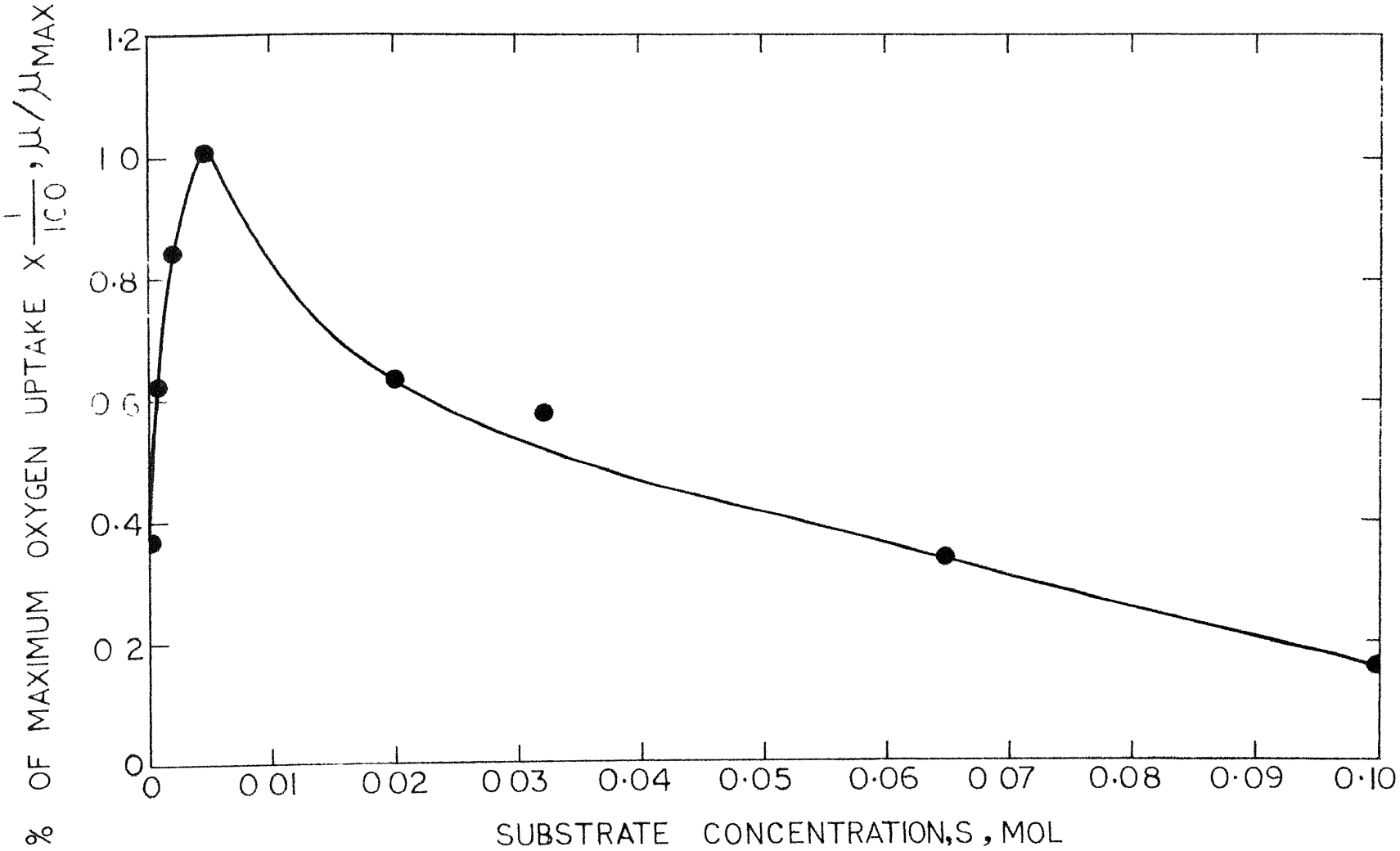
Before the above equations are treated any further it may be recalled that the specific growth rate constant, μ, has been described as a function of substrate concentration according to the following equation:

$$\mu = \mu_{max} \cdot \frac{S}{K_S + S} \qquad (10)$$

According to the data of Meyerhof (47) as plotted in Figure 26 in terms of percent of maximum respiration rate at various substrate concentrations, it is seen that the activity is maximum at 0.005 M substrate concentration and lower at other concentrations. Since the respiration activity can be taken as a direct indication of specific growth rate constant, the ordinate of the plot in Figure 26 can also be taken as the ratio of specific growth rate constant to maximum growth rate constant, μ/μ_{max}. Comparing equation (10) with this plot, it is seen that it cannot be applied in case of the present system. Based on Figure26 the variation in μ with respect to substrate, S, can be defined by the following equations for range of substrate concentration as shown.

$S \leq 0.005$ M NH_3-N., $\quad \frac{\mu}{\mu_{max}} = 1.05(1 - e^{-690\ S}) \qquad (23)$

$S_s \geq 0.005$ M NH_3-N., $\quad \frac{\mu}{\mu_{max}} = 1.147\ e^{-23\ S} \qquad (24)$



FIG·26. OXIDATION OF NH_3–N, AFTER MEYERHOF (47)

If equations (20) to (22) can be reduced to

$$\mu = \frac{X_2}{X_1} \cdot D \tag{25}$$

$$P = Y_{P/X} \cdot X_2 \tag{26}$$

$$S = S_o - \frac{1}{Y_{X/S}} \cdot X_2 \tag{27}$$

Combining equations (23) and (24) with above and rearranging,

For $S \leq 0.005$ as NH_3-N,

$$S = -\frac{1}{690} \ln \left(1 - \frac{X_2}{X_1} \cdot \frac{D}{\mu_{max} \cdot 1.05}\right) \tag{28}$$

For $S \geq 0.005$ as NH_3-N,

$$S = -\frac{1}{23} \ln \left(\frac{X_2}{X_1} \cdot \frac{D}{\mu_{max} \cdot 1.147}\right) \tag{29}$$

Also,

$$X_2 = Y_{X/S} (S_o - S) \tag{30}$$

$$P = Y_{P/S} (S_o - S) \tag{31}$$

As reviewed earlier, the ratio of NH_3-N oxidized to NO_2^--N to carbon synthesized is about 30 (34). If it is assumed that nitrogen content of the cell is 8% (30), the ratio of NH_3-N incorporated into the cell to those oxidized will be 1 : 200. This being a small fraction, the constant, $Y_{P/S}$, in equation (31) can be taken as equal to unity.

Since formation of cellular mass, X_2, is not of any importance in the present context, the constant, $Y_{X/S}$, in equation (30) was not evaluated.

To be able to predict the rate of formation of product, NO_2^--N, it is necessary that S in equation (31) be known under a given set of conditions. The unreacted substrate, S, going out of the system can be estimated from equations (28) and (29).

To evaluate μ_{max} for Nitrosomonas, an experiment on the growth of Nitrosomonas was conducted. In this case, a culture vessel was

inoculated with nitrifying seed and the substrate concentration was kept at 700 mg/l (0.05 M) NH_3-N. The pH of the liquid in the vessel was kept at 8.0 by adding phosphate buffer to it. Inorganic medium, as described earlier, was also provided along with a sand bed.

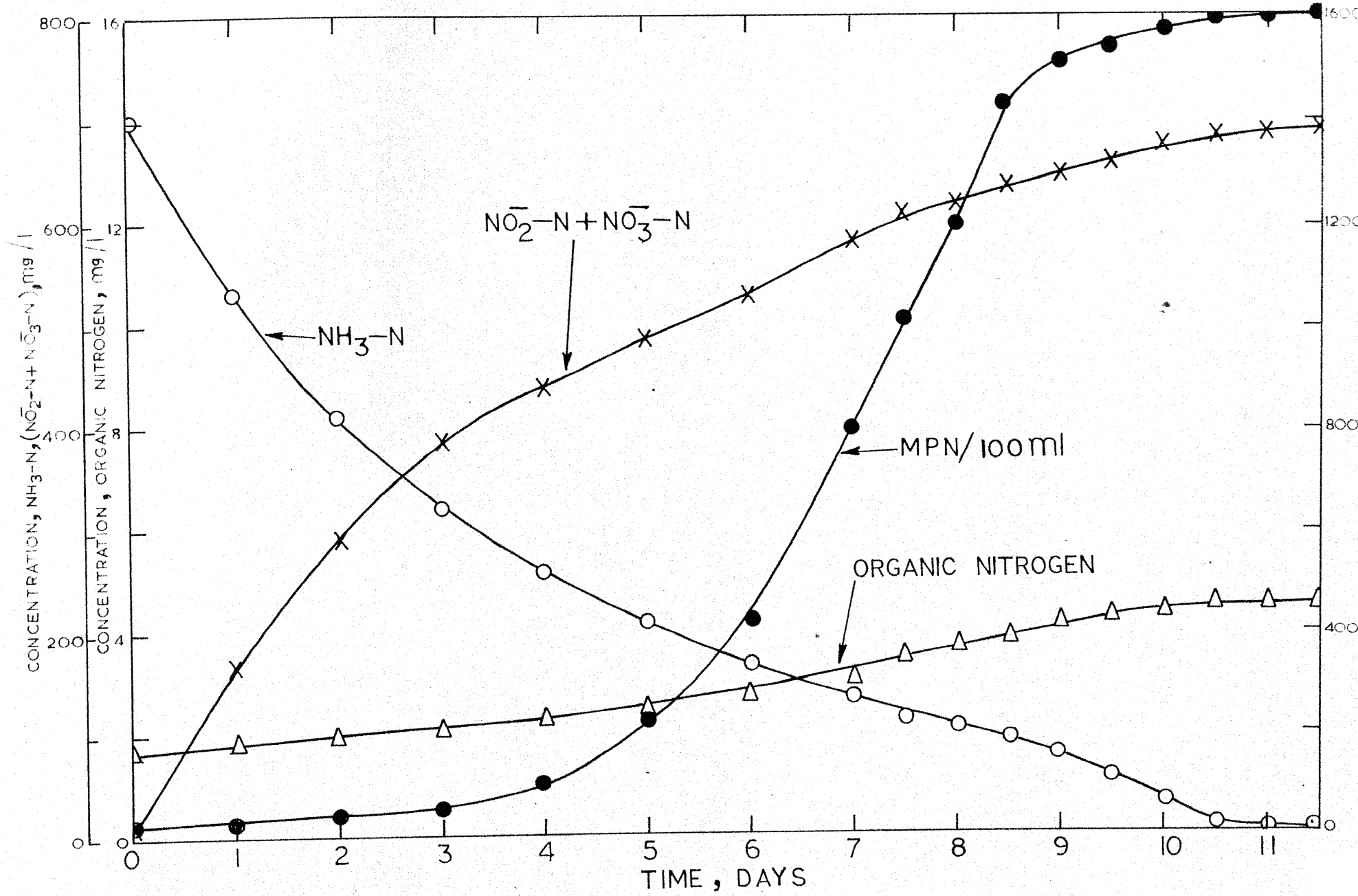
While drawing samples, the contents of the vessel were stirred vigorously so that the bacteria sticking to the sand bed be in suspension.

Figure 27 gives the plot of MPN/100 ml, NH_3-N, NO_2^--N, NO_3^--N, and organic nitrogen for daily analyses of samples drawn from the growth vessel containing 0.05 M NH_3-N as the substrate.

A comparison of the MPN/100ml values of the growth vessel as shown in Figure 27 to those of the continuous flow reactor as shown in Figure 28 reveals that the number of nitrifying bacteria is considerably higher in the former case. Although it is true that these two systems are different, yet it can be noted from this comparison that in the case of continuous flow reactor, the number of nitrifying bacteria found in suspension does not represent their total number present in the reactor.

With the data shown in Figure 27, Figure 29 has been prepared. From this, the value of μ_{max} comes out to be 0.121 day^{-1}. This value of μ_{max} seems to be logical since from Meyerhof's (47) data, μ_{max} has been found to occur at 0.005 M (70 mg/l) NH_3-N. This concentration of NH_3-N corresponds to a value of dilution rate, D, in the case of continuous flow reactor with varying hydraulic loadings, as 0.31 day^{-1}. Since μ_{max} is less than D_{max} as Y_2/Y_1 is a fraction in equation (25), the value of μ_{max} as 0.121 day^{-1} can be relied upon.

The unreacted substrate, S, going out of the reactor can be



FIG·27. GROWTH OF NITROSOMONAS WITH RESPECT TO NITRIFICATION

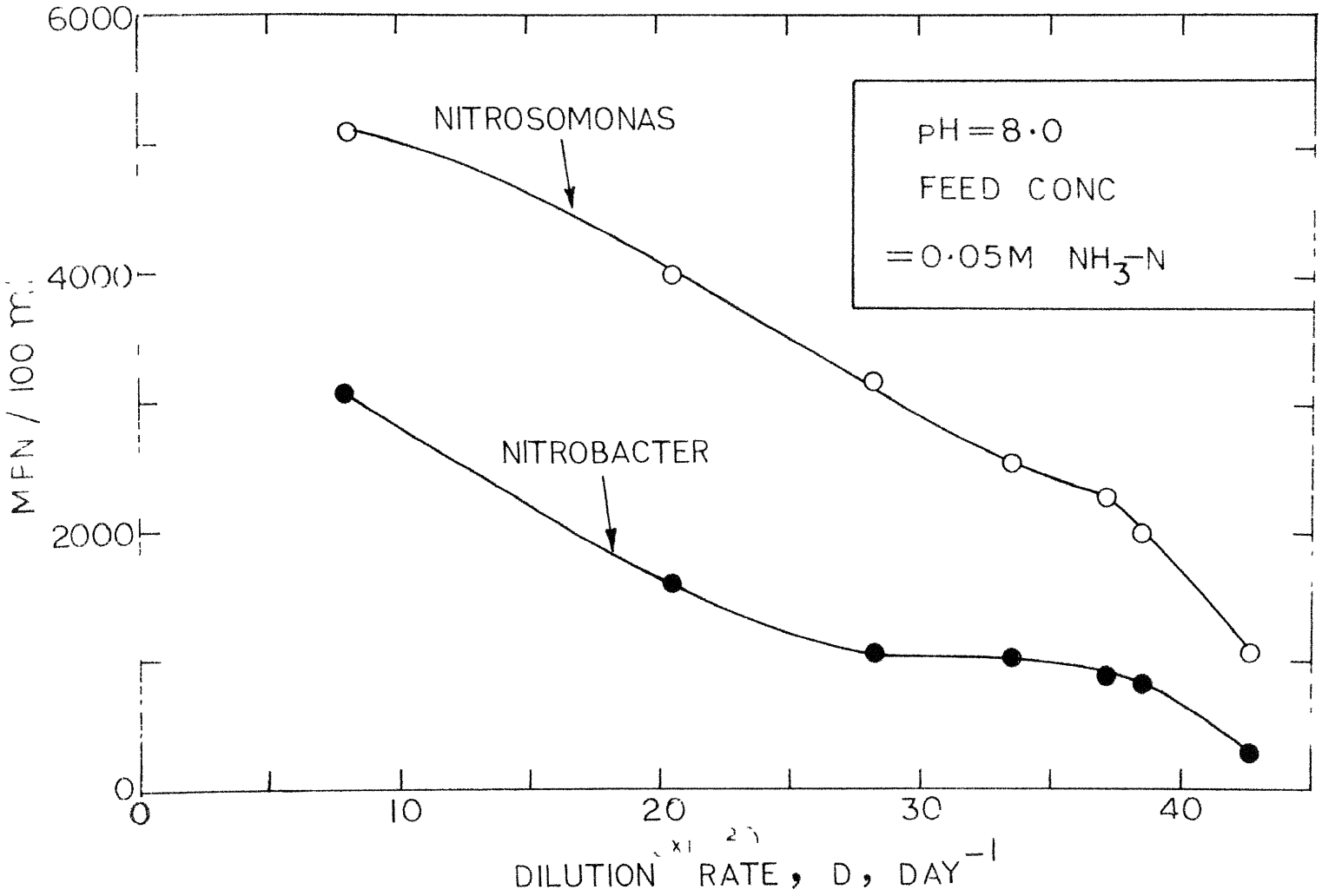
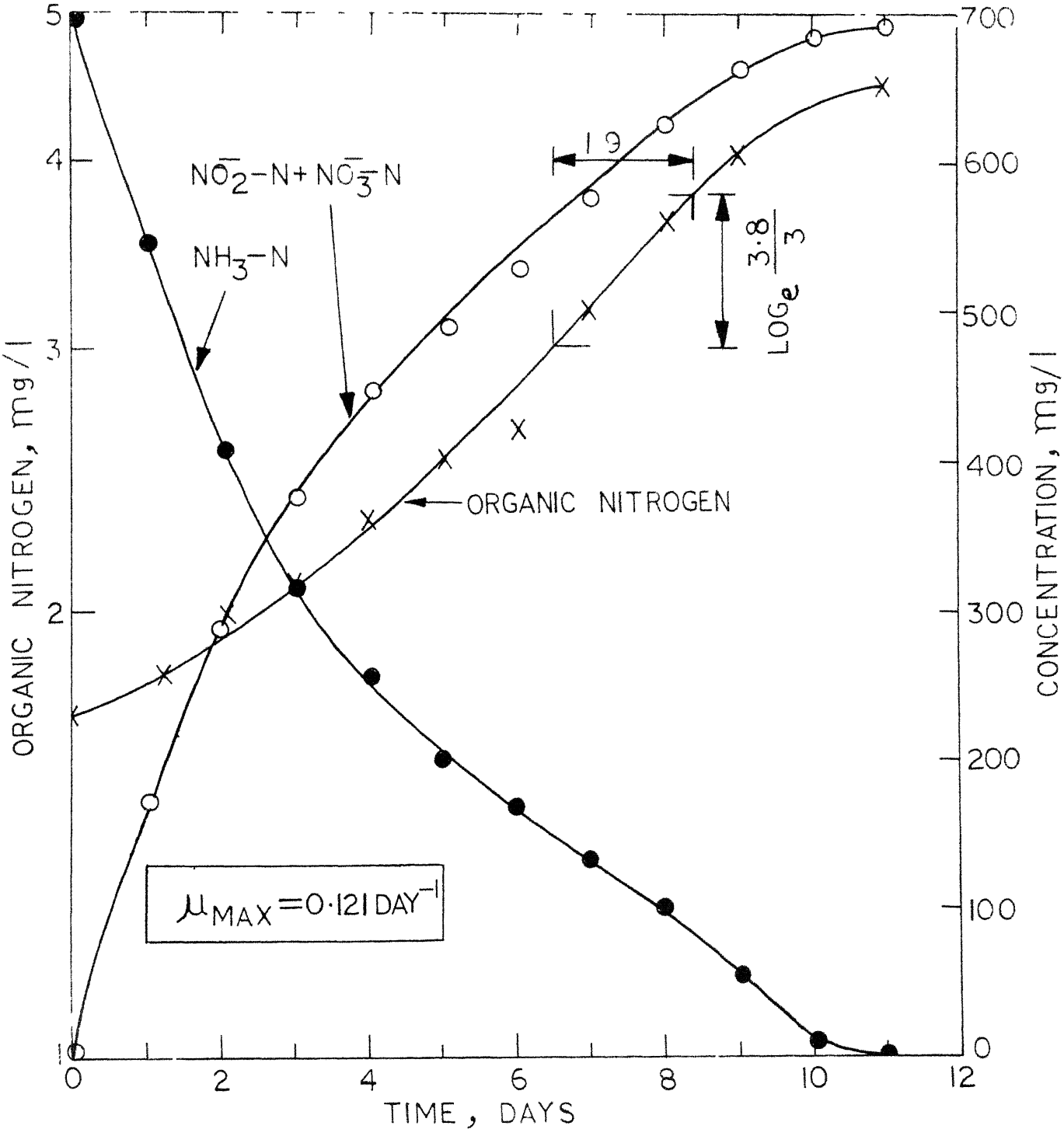


FIG 28 GROWTH CURVES OF NITROSOMONAS AND NITROBACTER IN THE CONTINUOUS-FLOW REACTOR



FIG·29. GROWTH CHARACTERISTICS OF NITROSOMONAS

estimated from equations (28) and (29).

The value of X_2/X_1 in equations (28) and (29) remains to be estimated. This value will be a function of dilution rate, D, surface area of the sand particles, and, the extent of agitation. For a given physical system, all these factors are constant except the dilution rate. Variation of X_2/X_1 with respect to D is calculated by equations (28) and (29) and the data of Figure 25, and plotted in Figure 30. For these calculations, the value of μ_{max} has been taken as 0.121 day^{-1}.

From the data shown in Figure 27, the values of N/C have been calculated considering that nitrogen, N, constitutes 8% of the organic content of the cell and the carbon content, C, of the organic matter is 50% (30). The values of N/C have been found to lie between 23 and 32. These values show good conformity to the values of N/C as 30 and 35 obtained by Baas-Becking and Parks (48) and Meyerhof (34) respectively.

With these values of N/C, energy relationships may be obtained. From the standpoint of free energy, with the values of free energy suggested by Clifton (30) and Stephenson (47), the free energy efficiency of Nitrosomonas lies between 9.4% and 6.4%. This gives good correlation to the free energy efficiency of Nitrosomonas as 5.9% which was found by Meyerhof (47). However, Hofmann and Lees (49) have pointed out that these calculations apply only to the stage of growth the culture reaches at the time of analyses are made and that, if old cultures are examined, then the values obtained may be low. In the case of Nitrosomonas, which Hofmann and Lees (49) examined, the efficiency of young cultures appears to be of the order of 40% whereas that of old cultures was 7%. Hence it may be assumed in the present case that the culture of Nitrosomonas was an old one which is evident from free energy efficiencies.

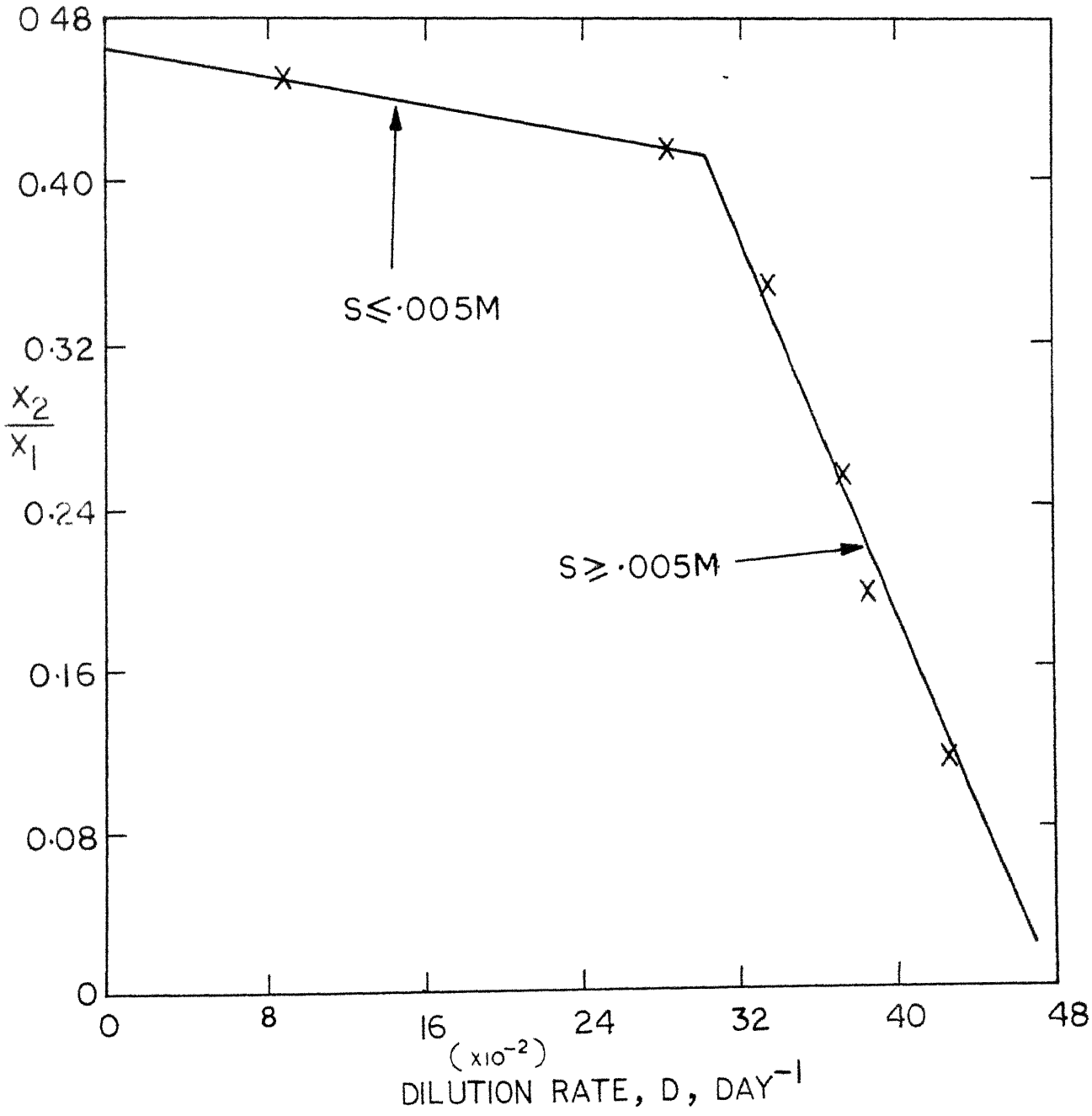


FIG. 30. PLOT OF $\frac{X_2}{X_1}$ VS D

3. Effect of substrate concentration

Equations (28) and (29) show that the substrate concentration, S, in the reactor is independent of the feed concentration, S_o, which is the concentration of NH_3-N in the influent. This would be true if all the bacteria present in the reactor would multiply. In this case as postulated earlier, only those microorganisms are capable of multiplication which are sticking to the sand particles. Since the surface of the sand bed is a limitation for a particular reactor, X_1 cannot increase indefinitely. As a result, the microorganisms would nitrify the influent NH_3-N and will account for same substrate concentration up to a particular concentration of influent NH_3-N when it reaches the saturation level. After this particular saturation level, the substrate concentration of the reactor would also start building up as the feed concentration increases.

The above is demonstrated by the results of the experiment in which different feed concentrations were tried. Figure 31 and Figure 32 show respectively how nitrification proceeded with time when varying doses of feed concentrations were tried keeping the flow-rate at 1060 ml/day and 640 ml/day, respectively.

With the values of NH_3-N, NO_2^--N, and NO_3^--N at equilibrium conditions obtained from Figures 31 and 32, Figure 33 has been prepared. From Figure 33, it is seen that S is independent of S_o up to a value of S_o equal to 700 mg/l for a flow-rate of 1060 ml/day and also up to a value of S_o equal to 950 mg/l when the flow-rate is 640 ml/day. Hence, it may be concluded that S is independent of S_o up to a certain value of S_o which may as well depend on the flow-rate.

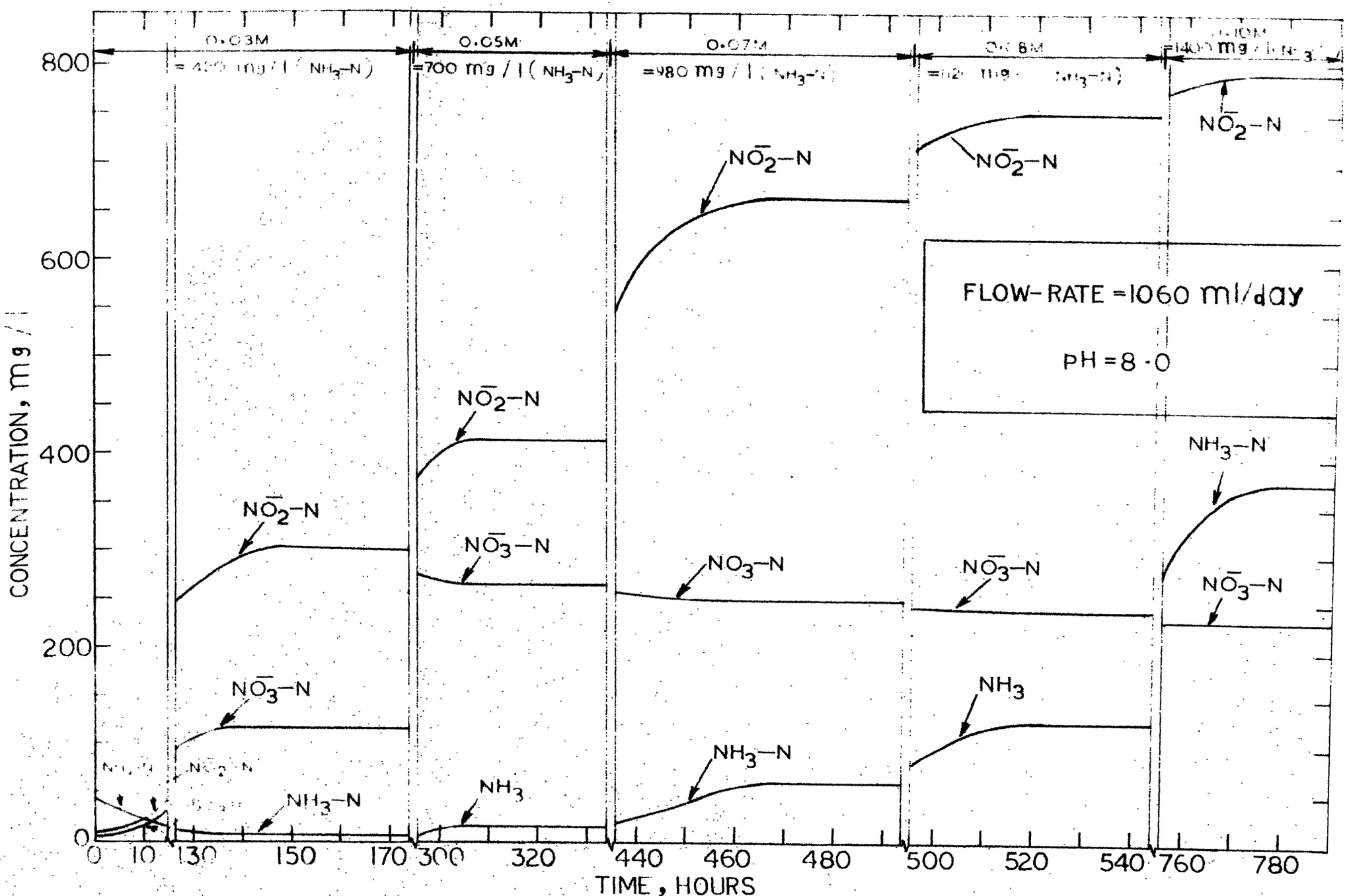


FIG. 31. RATE OF NITRIFICATION WITH RESPECT TO TIME FOR FEED CONCENTRATION VARYING FROM 0·03M TO 0·10M NH_3–N

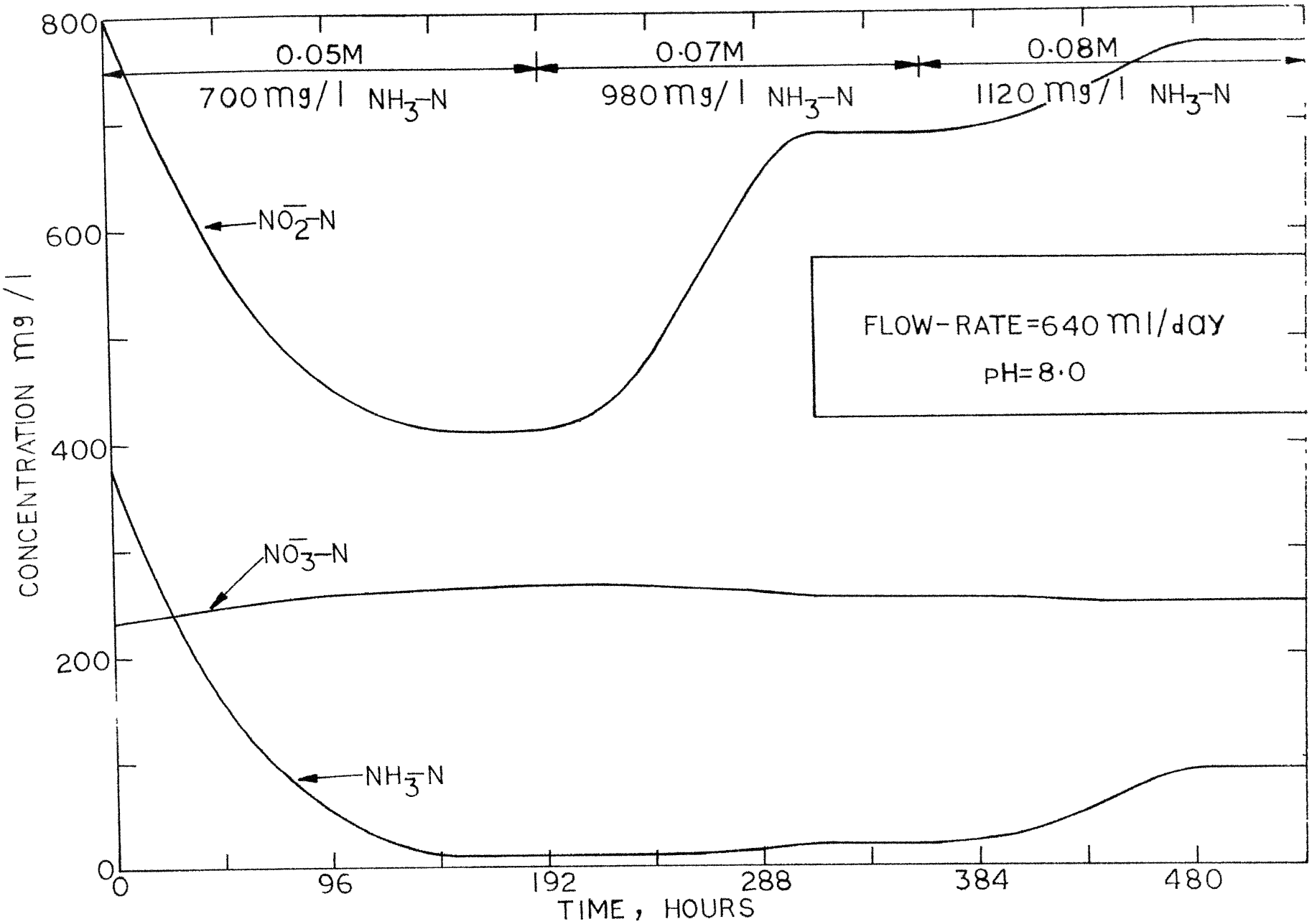


FIG. 32-NITRIFICATION WITH RESPECT TO FEED CONCENTRATION VARYING FROM 0·03M TO 0·08M NH_3–N

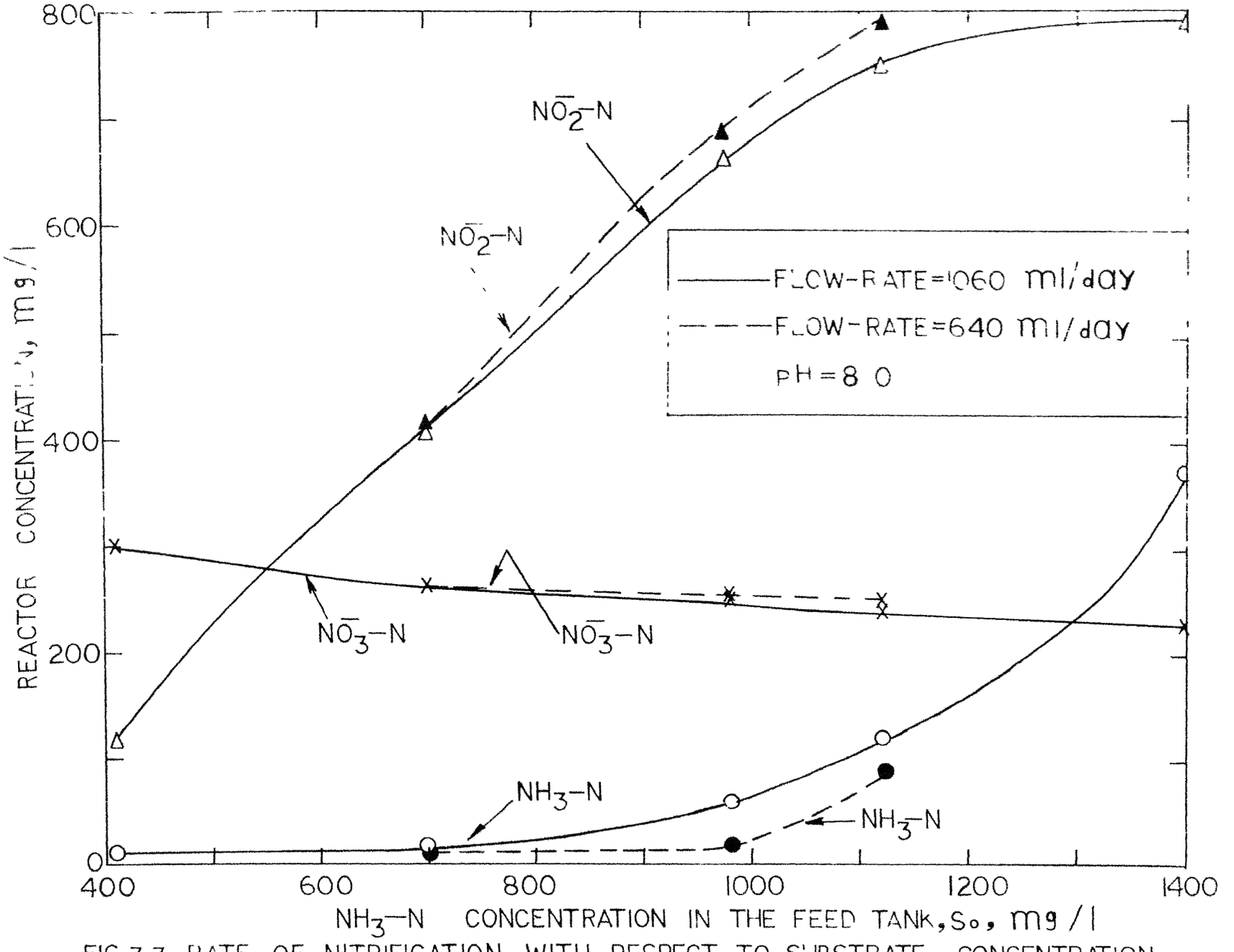


FIG.33. RATE OF NITRIFICATION WITH RESPECT TO SUBSTRATE CONCENTRATION

For citation's sake, a hypothetical example may be considered here.

Let certain fertilizer wastes contain 950 mg/l of NH_3-N and nitrification is desired to the extent of 98%. The flow-rate of the incoming wastes is 640 ml/day and the value of μ_{max} may be assumed to be 0.121 day^{-1}. The volume of the reactor is to be designed.

Since for a flow-rate of 640 ml/day, S is independent of S_0 up to a value of S_0 equal to 950 mg/l, hence in this case, S is independent of S_0.

Moreover, S is equal to 19 mg/l or 1.4×10^{-3} M. Therefore, in this case, equation (28) is to be employed.

Substituting the given values in equation (28) it is found that

$$D \cdot \frac{X_2}{X_1} = 0.07$$

With the help of Figure 30 and by trial and error method, the value of D comes out to be 0.16 day^{-1}.

Hence for a flow-rate of 640 ml/day, the volume of the reactor should be 4 liters.

VI. CONCLUSIONS

Based on the findings of this study on nitrification employing nitrifying column, culture vessel, and completely mixed continuous flow reactor, the following conclusions may be drawn:

1. In the beginning, oxidation of nitrite to nitrate is rather slow compared to oxidation of ammonia to nitrite. This can be attributed to a longer lag period for Nitrobacter compared to that of Nitrosomonas.
2. Both ammonia and nitrite at higher concentrations are responsible for inhibition of nitrifying organisms. Nitrosomonas are inhibited by 1400 mg/l (0.10 M) NH_3-N and Nitrobacter are inhibited by 964 mg/l (0.069 M) NO_2^--N. Nitrosomonas are also inhibited by 1100 mg/l (0.0799 M) NO_2^--N.
3. Changes in pH level affect the rate of nitrification considerably. Optimum pH range for Nitrosomonas lies between 7.8 and 8.3 whereas the optimum pH range for Nitrobacter lies between 8.0 and 8.5.
4. Nitrification can proceed even at extreme pH values like 5.5 or 9.5. However, there is a gradual fall in the rate of nitrification at pH values other than the optimum ones.
5. Provision of a sand bed in the reactor improves the rate of nitrification appreciably.
6. A hydraulic loading of 204 ml/(day)(l) for an influent concentration of 700 mg/l (0.05 M) NH_3-N accounts for nitrification to the extent of 95%. At higher hydraulic loadings, nitrification becomes incomplete.
7. The value of the maximum growth rate constant, μ_{max}, may be obtained for a particular value of μ and for a known substrate concentration, S, by the following relationships:

For $S \leq 0.005$ M NH_3-N,

$$\mu = \mu_{max} \cdot 1.05 \, (1 - e^{-690 \, S}) \qquad (23)$$

For $S \geq 0.005$ M NH_3-N,

$$\mu = \mu_{max} \cdot 1.147 \, e^{-23 \, S} \qquad (24)$$

8. The value of μ_{max} for a substrate concentration of 0.05 M NH_3-N is 0.121 day^{-1}. The value of D_{max} is higher than that of μ_{max} as X_2/X_1 in the following equation is a fraction.

$$\mu = D \cdot X_2/X_1 \qquad (25)$$

9. The value of N/C for Nitrosomonas has been found to vary betwqen 23 and 32.

10. The free energy efficiency of Nitrosomonas lies between 6.4% and 9.4%.

11. Surface of the sand bed for a particular reactor is limited and, hence, X_1 cannot increase indefinitely. The microorganisms may account for same substrate concentration in the reactor up to a particular influent concentration of NH_3-N and up to the saturation level of the sand bed. After this, substrate concentration in the reactor starts building up as the feed concentration increasss.

12. S is independent of S_0 up to a certain value of S_0. This limit again depends on the flow-rate or hydraulic loading. The lesser the flow-rate, the higher will be the value of S_0 up to which S is independent of S_0. This value of S_0 is 700 mg/l and 950 mg/l for flow-rates of 1060 ml/day and 640 ml/day, respectively, when the reactor volume is 5 l.

VII. SUGGESTIONS FOR FUTURE WORK

It has been emphasized before that waste treatment by the nitrification-denitrification system has not yet gained popularity and admiration so far. As a result of this, a considerable amount of extensive and exploratory research is needed in this field before such a system could be clearly and fundamentally well defined.

However, in view of the conclusions arrived at from the present study on nitrification, the following considerations seem important enough to warrant extension of the study:

1. Studies should be undertaken to evaluate further relationship with respect to temperature. It is although true that nitrifying bacteria are mesophilic in character and will be well developed in normal temperature zones which may range from 20°C to 35°C. But in extreme climatic conditions, a study of effect of temperature on the rate of nitrification may lead to optimization of temperature and thereby suggest the possible temperature zone for active nitrification.
2. Studies should be carried out to assess the relationship of rate of aeration with the rate of nitrification. It is presumed, however, that the relationship may be linear up to certain extent, and thereafter, greater supply of oxygen may not enhance the rate of nitrification which has attained the maximum value.
3. Studies should be carried out to find whether an appreciable nitrate accumulation in the system inhibits or retards nitrification as nitrate will be accumulating with the progress of nitrification.
4. Studies should be initiated to find the best shape and size of the reactor vessel. Determination of effective depth is important in this case as the nitrifying bacteria develop only in aerobic conditions

and as such, provision of shallow depth may prove to be beneficial. Moreover, it has been found that sewage kept under quiscent conditions in shallow layers may reduce the time of nitrification.

5. An attempt should be made to find out the thickness of the sand bed for its highest effectiveness. Moreover, it is presumed that replacement of such a layer after some time may enhance nitrification and, hence, an appraisal of this time interval between two replacements may be fully appreciated.

6. An investigation may be carried out to find the values of X_2/X_1 for corresponding values of dilution rate, D, of the reactor. In that case, the value of μ_{max} may be directly obtained from equation (25).

7. Present study has been based with pure NH_4Cl solutions. Since the fertilizer wastes may contain other foreign materials, all the findings of the present study may be evaluated in terms of different fertilizers available in the market.

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